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Lifecycle Efficiency of Mice Divergently Selected for Heat Loss

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LIFECYCLE EFFICIENCY OF MICE DIVERGENTLY SELECTED FOR HEAT LOSS

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Divergent selection for heat loss as an indication of maintenance energy requirements in mice resulted in a low (ML) and high maintenance line (MH), and an intermediate control line (MC). Improved feed efficiency has been observed in ML mice, which would be economically beneficial in livestock species. However, previous work showed evidence of unfavorable correlated responses to selection in ML mice compared to MH mice. Therefore, the objective of this study was to model a lifecycle similar to a livestock production system using these lines and determine if these correlated responses diminish the benefit of improved feed efficiency by calculating lifecycle efficiency. Feed intake, reproductive performance, productivity, and body composition were recorded on 63 mating pairs and sampled offspring from each line. Values from mixed-model analysis of these data were used to calculate total lean output of offspring and culled mating pairs and total energy intake of offspring and mating pairs. Lifecycle efficiency was calculated as the sum of offspring and parent output divided by the sum of offspring and parent input. Pairs were cohabitated at 7 wk of age and maintained for up to 1 yr unless culled and offspring were maintained from 21 to 49 d of age. Survival was measured as number of parities achieved by a mating pair. Hypothetical parity distribution of 100 mice at equilibrium was calculated using survival analysis results. High maintenance mice consumed 7 to 20% more feed for maintenance than ML mice. Control mice had greater body weights, total numbers weaned and weaning weights compared to selected animals,

while there were no significant differences between MH and ML mice for any traits. Control mice had greater survival rates overall, though ML mice had greatest survival rates up 5 parities, while MH mice had greater survival rates in later parities. Although ML mice were more efficient than MH mice, they were nearly identical to MC mice due to poorer productivity. Differences in body weight and numbers weaned had the greatest effect on lifecycle efficiency, therefore selection for reduced maintenance energy requirement should be utilized as part of selection indices or in terminal crosses.

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Introduction

Presently, feed costs represent the largest economic input in livestock production systems, therefore profitability of these systems can be improved by reducing feed intake (Ferrell and Jenkins, 1985; Noblet et al., 1993). However, livestock species must still produce sufficient product, and limiting overall feed intake could potentially reduce growth or production of milk, eggs, etc. Therefore, improving feed efficiency is likely a more advantageous route. Feed efficiency has traditionally been measured as a ratio of gain to feed (or the inverse), but selection to improve feed efficiency as defined by this ratio is not particularly effective. Additionally, the nature of this measurement still does not necessarily allow for reduction of feed intake independent of growth.

In the study of energy metabolism, energy intake is partitioned between several functions including maintenance and growth or production. Maintenance energy requirements encompass the amount of energy input required to maintain basic metabolic functions while maintaining a constant body weight (Williams and Jenkins, 2003). Metabolizable energy consumed to meet maintenance energy requirements represents the largest proportion of total feed intake, and maintenance energy requirements are more variable than those of gain or production of output (Ferrell and Jenkins, 1985). This implies larger genetic variation which suggests the possibility of utilizing selection to lower maintenance energy requirements, thereby lowering feed intake. Additionally, reducing maintenance energy requirements independent of energy necessary for production should improve feed efficiency as growth and production should be unaffected.

Improving feed efficiency through genetic selection is a current priority for livestock species. There are multiple ways to measure feed efficiency, and previous selection has attempted to select for increased growth or utilize ratios of feed and gain as a selection criterion. These methods contain both statistical and biological difficulties and may not be the most effective choice to achieve genetic gain. More recently, many studies have used residual feed intake (RFI) as a selection trait as its calculation is designed to predict the amount of feed consumed over what is required for maintenance (and growth or production in some instances) (Koch et al., 1963). This method is still imperfect and can be variable depending upon how maintenance requirements are calculated. Attempts to isolate and select for reduced maintenance energy requirements and capture correlated responses in feed intake and efficiency may be more suitable. Long-term selection experiments for RFI or maintenance energy requirements have been performed with positive results in swine and poultry, and improvement in RFI has been observed with one generation of selection in beef cattle (Gilbert et al., 2006; Luiting et al., 1991; Richardson and Herd, 2004).

As with many traits, selection for maintenance energy could cause correlated responses in traits other than feed intake, which could be either beneficial or detrimental. Though selection should only affect maintenance energy requirements if implemented accurately, costs of production and gain are often correlated and could also be affected. Selection could alter patterns of protein versus lean deposition, resulting in fatter animals, which is undesirable for livestock species. Reducing maintenance energy requirements could also negatively affect reproductive performance or overall survival rates, increasing replacement rates and reducing the efficiency of the system.

To further explore the effect of using selection to reduce maintenance energy requirements, Nielsen et al. (1997b) used direct calorimetry to create two lines of mice divergently selected for maintenance requirements, using heat loss as an indicator trait. Selection proved effective in both directions, which was maintained during relaxed selection and still produced a response when selection was renewed. As expected, the low heat loss line consumed significantly less feed than the high line. However, the low line tended to have a higher fat percentage and showed evidence of poorer reproductive performance, which could counteract the benefits of reduced feed intake in the overall production system (McDonald and Nielsen, 2007; Nielsen et al., 1997a). Furthermore, feed intake and reproductive measures have previously only been measured at a single time point and have not been evaluated across all life stages. Also, survival rates in these lines are unknown and could have ramifications on overall efficiency of these lines. Therefore, it is necessary to integrate correlated effects and evaluate these lines in terms of lifecycle efficiency to determine if the reduction in feed intake associated with lowering maintenance costs is maintained through all life stages and outweighs any detrimental responses to selection.

CHAPTER I

Maintenance energy

The metabolizable energy (ME) consumed by livestock species can theoretically be partitioned into several components, the simplest of which separates ME into heat energy and recovered energy. Heat energy refers to the portion of energy released as heat, while recovered energy is the energy retained by the body in the form of fat or protein deposition, or utilized to form useful product (milk, eggs, etc.). In livestock production systems, the products of recovered energy are the source of revenue while heat energy is a necessary input, it is unrecoverable in terms of profit. Additionally, it is estimated to account for 70 to 75% of total ME intake in beef cattle (Ferrell and Jenkins, 1985). Therefore, much of the focus of energetic research focuses on further partitioning heat energy into physiological components that can perhaps be reduced through various methods (Williams and Jenkins, 2003).

Heat energy can be partitioned into fasting heat production and heat increment. Fasting heat production is the heat production of animals in a post-absorptive state, measured after a period of fasting. Heat increment includes heat production associated with maintenance functions (digestion, fermentation, waste formation) and heat of product formation. Fasting heat production and heat increment associated with maintenance functions can be combined and referred to as maintenance energy requirements. Maintenance is defined as the amount of energy intake required for an animal to maintain body weight (no gain or loss of weight). Heat energy can be measured as heat production by direct or indirect calorimetry, and subsequently differences in maintenance energy requirements of individual animals can be estimated.

If animals are measured at maturity, (when they are not gaining or producing product) the heat of product formation can be assumed to be close to zero, and therefore heat production is estimating maintenance energy requirements. If animals are fasted before measurements, the heat increment portion is presumably close to zero and can be ignored, providing estimates of fasting heat production (Williams and Jenkins, 2003).

Variation in maintenance energy requirements

One way to exploit this partitioning of energy requirements is through selection to reduce maintenance energy requirements. However, in order for selection to be successful, sufficient genetic variation in maintenance energy requirements must exist. Ferrell and Jenkins (1985) concluded that there was considerable variation in energy expenditure and the largest proportion of total expenditure was due to maintenance functions, particularly metabolism of visceral organs. A study of monozygotic twin cattle found significantly higher variation between pairs versus within pairs, indicating a large genetic component of variation (Hotovy et al., 1991). Authors also estimated a heritability of 0.52 for maintenance energy requirements. Several other studies have also found evidence for genetic variation in maintenance energy requirements, and indicate that the variation in maintenance energy requirements is higher than the variation in production energy requirements (Derno et al., 2005; Herd and Arthur, 2009; Herd et al., 2004). Thus, maintenance energy requirement has sufficient genetic variation and can likely be altered through selection. Maintenance energy itself is a difficult trait to measure and therefore generally not included as a direct selection criterion. Instead,

selection for feed intake and growth rate, feed and gain ratios, or residual feed intake is utilized to attempt to reduce the maintenance energy component of total energy intake.

Selection to improve feed efficiency

Selection for growth and feed intake

Several experiments evaluating selection for rate of gain or feed intake have been conducted in mice as well as livestock species. Sutherland et al. (1970) developed mouse lines that underwent 21 generations of selection for rate of gain or 9 generations of selection for rate of gain followed by 12 generations of selection for reduced feed intake. During the initial 9 generations of selection for gain, both gain and feed intake increased in both lines. Selection differentials for gain were similar (22 and 24 g for the growth only and growth and feed intake line, respectively) but secondary selection differentials for feed intake were considerably different (27 and 47 g for the growth only and growth and feed intake line, respectively). However, in the second phase of selection, the line selected for feed intake had a selection differential of 186 g for intake, while the line selected for gain only had a selection differential of 111 g. Both lines had similar selection differentials for gain in the second period of selection (27 and 31 g for the growth only and growth and feed intake line, respectively). Feed efficiency, measured as gain:feed improved in both selection lines (secondary selection differentials of 0.1314 and 0.1501, for growth only and growth and feed intake lines, respectively). Authors determined that selection for increased rate of gain only moderately increased feed intake and therefore resulted in improved feed efficiency, and found a genetic correlation of 0.91 between gain and feed efficiency.

Selection for lean gain applied for 5 generations in rats was evaluated by Notter et al. (1976) by measuring protein gain from 3 to 9 weeks of age. Wang and Dickerson (1984) evaluated the same population of rats after it had undergone 14 total generations of selection. In the first 5 generations of selection, total body weight gain and protein gain both tended to increase, and had increased significantly after 14 generations of selection when compared to a control line (25% heavier body weights and 20% greater protein gain). After 14 generations, the selection line also showed increased feed intake, but had improved feed efficiency compared to control animals (12% more daily feed intake and 13% more efficiency) (Wang and Dickerson, 1984).

Klein et al. (1999) developed lines selected for increased body weight or protein, as well as a control line. The increased body weight line had a higher metabolizable energy intake than the protein line, and both selection lines consumed more than the control line. Similarly, the body weight line had greater body weights than the protein line, and both were heavier than the control line. This resulted in no change in feed efficiency in the protein line, and reduced feed efficiency in the body weight line.

Castro Bulle et al. (2007) evaluated feed efficiency in steers from a cattle population that had undergone selection for high postweaning gain for over 15 yr. The high growth line consumed more feed per day and had a higher growth rate than the low growth line. Protein and fat gain were both higher in the high growth line as well. Additionally, the high growth line had a greater gain to feed ratio and thus better feed efficiency. The high growth line had a lower initial body weight but a higher final body weight compared to the low growth line.

Selection for increased gain and selection for reduced feed intake have both been shown to be effective in multiple species. However, selection for increased gain alone does seem to increase feed intake, and even though an improved feed to gain ratio is observed, this selection method requires further investigation due to the economic cost of increased feed intake. Selection for increased gain should likely not be implemented independent of selection for reduced feed intake.

Selection for feed efficiency or feed conversion

Sutherland et al. (1970) selected mice for feed efficiency (gain/feed) for 12 generations after an initial 9 generations of selection for total gain only. Mice showed notable increases in gain per generation paired with small increases in feed intake. Therefore, selection was successful in improving feed efficiency. However, the realized heritability for feed efficiency was low (0.17).

Notter et al. (1976) and Wang and Dickerson (1984) evaluated the same population of rats selected across 5 and 14 generations of selection, respectively, for efficiency of lean gain. Efficiency of lean gain was measured as protein gain from 3 to 9 wk of age divided by feed intake. No differences in gain, feed intake, or feed efficiency were seen after 5 generations of selection, but after 14 generations, the selection line had a 15% increase in protein gains with only a 5% increase in feed intake, resulting in an 11% improvement in feed efficiency compared to the control line.

Hastings et al. (1997) essentially selected mice for feed efficiency by selecting for feed intake corrected by phenotypic regression on mean body weight (in essence, the authors created a selection index balancing feed intake and body weight). The high line

consumed 35 to 45% more total feed than the low line, and 70 to 90% more feed when adjusted for body weight. The high line also weighed 7 to 11% more than the low line. Thus, the high line had a less desirable feed efficiency than the low line.

Holder et al. (1999) developed several feed efficiency lines in mice, each selected based on slightly different criteria: a gain deviation line selected for greatest gain adjusted to a constant intake, an intake deviation line selected for least intake adjusted to constant gain, an intrinsic efficiency line selected for least intake adjusted to a constant gain and adjusted for the average weight maintained during the period, a line selected for the traditional gain to feed ratio, and a control line. Because the gain to feed ratio generally has a very low heritability (0.00 in this study), the goal was to evaluate other methods of selecting for improved feed efficiency. The gain deviation line did not differ in either intake or gain from the control line, but did have a high and positive genetic correlation with gain:feed (0.83). However, it also had a low heritability (0.04). The intake efficiency line showed reduced feed intake with no change in gain. While it had a moderate heritability (0.35), the genetic correlation with gain:feed was very low (0.01). The intrinsic efficiency line had both a moderate heritability (0.28) and genetic correlation with gain:feed (0.21), and also showed reduced feed intake with no change in gain.

Jungst et al. (1981) practiced selection for feed conversion (feed:gain) in Yorkshire boars for five generations. Feed conversion showed a change of -0.019 per generation, which represents only a slight improvement. Rate of gain increased by 0.003 kg/d per generation, and daily feed intake decreased by 0.007 kg per generation.

Realized heritability for feed efficiency was 0.09. None of these coefficients were significantly different from zero, indicating that direct selection for feed efficiency was ineffective.

Attempts to improve feed efficiency by selecting for a gain to feed ratio (or the inverse) have only been shown to be moderately successful. Generally, these traits have very low heritabilities and selection based on a ratio is problematic. However, selection on an index of feed intake and growth was shown to be much more productive.

Selection for residual feed intake

The concept of residual feed intake (RFI) to measure feed efficiency is commonly attributed to Koch et al. (1963), though Byerly (1941) first untilized it in poultry. RFI has recently been gaining traction in the livestock industry. Calculation involves adjusting total feed intake for body weight and gain in order to partition intake into expected feed for maintenance and observed gain and a residual portion. The residual portion (total feed intake subtracted from expected feed intake) is referred to as RFI and animals with a higher (positive) RFI are considered inefficient when compared to those with low (negative) RFI. RFI can also be adjusted for other measures, and backfat is commonly included in calculation of expected feed intake in swine.

RFI was first fully implemented as a selection criterion in poultry. Luiting et al. (1991) developed selection lines for high and low RFI (referred to as residual feed consumption) in White Leghorns. A larger feed conversion ratio and RFI was observed in the high RFI line compared to the low RFI line, indicating a poorer feed efficiency in

animals with greater RFI. There were minimal differences in daily body weight gain and mean metabolic body weight.

Selection for a single generation for RFI was practiced in beef cattle and progeny of the low RFI parents showed a lower RFI than progeny of the high RFI parents (Richardson and Herd, 2004; Richardson et al., 2001). The high RFI steers consumed 5% more feed per day than low RFI steers, while average daily gain and final body weight did not differ. The traditional feed conversion ratio was not different between low and high RFI steers.

Two groups have practiced selection for RFI in pigs with successful results. Cai et al. (2008) selected Yorkshire pigs for reduced RFI, along with a control line. Selection reduced RFI by 93 g/d after 4 generations of selection. Selection also resulted in a correlated reduction in average daily feed intake (about 165 g/d) and a 33 g/d reduction in average daily gain, resulting in an improvement of 1.36% feed efficiency after 4 generations of selection. Gilbert et al. (2007; 2006) practiced selection for RFI in both directions for 4 generations, creating a high and low RFI line. Lines differed in RFI by 0.3 standard deviations after 3 generation. There was no difference in average daily gain and daily feed intake was decreased in the low RFI line at both generations 2 and 3 of selection. This resulted in improved feed conversion rate for the low RFI line.

Selection for RFI has effectively been shown to reduce feed intake and improve feed efficiency in multiple species. In most cases, growth and other measures of production were not severely affected. RFI still has some computational and biological concerns that should be addressed. The calculation of RFI often varies across studies and

different methods can greatly influence results. Additionally, the idea of RFI is to reduce maintenance energy requirements and this result should be validated.

Relationship between feed efficiency and maintenance energy requirements

Mice

Direct selection for maintenance energy requirements would be difficult; however the relationship between selection for feed efficiency and maintenance energy has been explored in several species. Archer and Pitchford (1996) explored the phenotypic relationship between residual feed intake and maintenance energy requirements in mice. Maintenance energy requirements were estimated using growth and feed intake curves from data in individuals 3 to 18 wk of age by dividing the estimate of mature feed intake by the estimate for mature body weight or the estimate for mature body weight to the power of 0.75. Maintenance energy requirements were lowly correlated with RFI in mice less 7 wk of age, but highly and positively correlated in older mice, indicating that mice with high RFI, or poor feed efficiency, had higher maintenance energy requirements. Correlations with body composition also indicate that leaner mice have higher RFI and higher maintenance energy requirements. Johnston et al. (2007) measured basal metabolic rate (BMR), measured by oxygen consumption and carbon dioxide production and reproductive performance in mice. There was no relationship between BMR and reproductive success, litter size, litter mass, or pup mass that was not otherwise associated with body mass of the dam.

Wang and Dickerson (1984) selected rats for rate or efficiency of lean gain and measured heat production via direct calorimetry in both lines. Measurements

demonstrated a reduction in basal metabolism after 14 generations of selection in both lines (26.4 cal/g$^{.75}$/h for rate of gain and 26.1 cal/g$^{.75}$/h for efficiency of gain, compared to 28.4 cal/g$^{.75}$/h in the control). Mice from the rate of gain line required 9% less feed intake for maintenance while those from the efficiency of gain line required 10% less.

Klein et al. (1999) selected mice for increased body weight or protein content, as well as a control line and analyzed various energy metabolism parameters. Carbon dioxide production and oxygen consumption were measured and used to estimate heat production. Maintenance requirements of metabolizable energy were estimated by correcting metabolizable energy for protein and fat deposition and resting metabolic rate was estimated by regression of total heat production. Total heat production was higher in the protein line than the line selected for body weight alone (887, 831, and 846 kJ/kg·BW$^{0.75}$, respectively), and both had higher heat production compared to the control (791 kj/kg·BW$^{0.75}$). Maintenance energy requirements were also higher in the protein line (13 to 22%) than the body weight line, and both lines had higher requirements than the control line. The resting metabolic rate was lower in both selection lines when compared to the control line.

Selman et al. (2001) evaluated resting metabolic rate in a population of mice that had been selected for high or low feed intake corrected for body mass. Resting metabolic rate was evaluated as the rate of oxygen consumption measured using a respirometry system. In both males and females, resting metabolic rate was higher in high feed intake animals compared to low intake animals. Differences were explained in part by larger organ masses (particularly larger liver mass) in animals with high resting metabolic rates.

Poultry

Skinner-Noble and Teeter (2003) evaluated total heat production and basal metabolic rate in broilers classified as having good or poor feed conversion rates. Metabolic chambers measuring oxygen consumption and carbon dioxide production were used to estimate basal metabolic rate and heat production. Apparent metabolizable energy was calculated as the difference between the gross energy of feed consumed and the gross energy of excreta. Then the net energy for maintenance was calculated as hourly heat production multiplied by length of feeding period. There was no difference in total heat production, basal metabolic rate, or net energy for maintenance between good and poor feed converters. Authors suggest that lack of differences could be due to higher starting body weights in good converters and thus a higher amount of heat production is necessary to maintain that additional weight. Differences in body composition between good and poor converters could also be accountable. However, it is possible that basal metabolic rate does not differ due to feed efficiency in this population.

Luiting et al. (1991) explored metabolic differences in laying hens selected for high and low RFI. Heat production was determined from carbon dioxide and oxygen exchange. Simultaneous measurements of activity were measured by radar activity meters to determine heat production in the absence of activity (zero-activity heat production). There was a 64 to 147 $kJ/kg^{0.75}$ difference in total heat production between low and high RFI hens, with the high RFI line showing higher heat production. There were no differences between lines for zero activity heat production or heat production related to activity.

Gabarrou et al. (1998) also measured heat production via oxygen consumption and carbon dioxide production in laying hens selected for high and low RFI. Heat production was measured in both fasting and fed states, and the difference was used to estimate the heat increment of feeding. Fasting heat production was not different by line; however, fed heat production was 32% higher in high RFI hens. Heat increment of feeding was also higher in the high RFI line. When hens were fed 70%, 100%, and 130% of their *ad libitum* feed intake, heat production and heat increment were greater in high RFI hens and heat production increased with intake in high RFI birds, while it remained unchanged in the low line. Authors determined that differences in heat production were due to increased feed intake.

Swine

Barea et al. (2010) established high and low RFI line in French Large White pigs by practicing divergent selection for over 6 generations. Castrated males from these lines were chosen and evaluated for energy utilization measured in respiration chambers. Fasting heat production was measured after 1 day of fasting, followed by heat production measurements during 3 phases of growth (25 to 45 kg, 45 to 65 kg, and 65 to 95 kg). The low RFI pigs produced 8% less total heat production than the high line, largely due to a 10% reduction in fasting heat production. When total heat production and fasting heat production were adjusted to a constant retained energy, the differences were confirmed (a 6% and 9% difference in total heat production and fasting heat production, respectively). Additionally, the high RFI line had a greater maintenance energy requirement than the

low line, where maintenance energy requirements were measured as fasting heat production divided by the ratio of net energy to metabolizable energy (a 10% difference).

Beef cattle

Nkrumah et al. (2006) ranked beef steers by RFI into a high, medium, and low RFI group and measured oxygen consumption to estimate heat production and other energy metabolism parameters. Daily heat production was highly associated with RFI, and the low RFI steers had 21% lower heat production than the high group and 10% lower that the medium RFI group. Retained energy was highest in the low RFI group where the retained energy was 44% lower in the high group and 23% lower in the medium group. Heat increment of feeding was not different among the RFI groups.

Maintenance energy requirements were calculated in beef cattle selected for high growth for over 15 yrs by linear regression between retained energy and metabolizable energy intake. Retained energy and metabolizable energy intake were higher in the high growth line, but the maintenance energy requirement did not differ by line. Additionally, when animals were reclassified by RFI, maintenance energy requirements did not differ between high and low RFI steers (Castro Bulle et al., 2007).

Summary

Excluding data from poultry studies, selection for improve feed efficiency does appear to be associated with a decrease in heat production and maintenance energy requirements. The lack of this relationship in poultry could be associated with basic biological differences between mammals and avian species. However, it seems that

reducing maintenance energy requirements, measured by heat production, is associated with improved feed efficiency and should be further explored to determine the potential for altering maintenance energy requirements by selection.

Correlated responses to selection for feed efficiency

Body composition

Biondini et al. (1968) evaluated body composition (moisture, crude protein, ash, and ether extract) in mice selected for gain for 10 generations in 3 lines. Fat content measured by ether extraction increased proportionately much more than any of the other components in 2 of the lines.

In rats selected for rapid lean gain, the protein proportion of the carcass increased during the first 5 generations of selection, but decreased after 14 generations of selection had been applied. In rats selected for efficiency of lean growth, there were no significant differences in lean composition of carcasses after either 5 or 14 generations of selection (Notter et al., 1976; Wang and Dickerson, 1984).

Mice selected for feed intake adjusted by phenotypic regression on mean body weight also showed differences in predicted fat content (Hastings et al., 1997). The low intake line had the highest predicted fat content, while the high line had the lowest. However, the difference was about 2.4%, which was not significant. Line differences were higher (7.2%) in older animals (17 wk vs 10 wk of age), indicating that divergence may increase with age.

Klein et al. (1999) selected mice for increased body weight which resulted in a greater fat percentage, but no change in protein percentage when compared to the control

line. However, when selected for increased protein, there was a slight increase in protein percentage compared to the body weight line and no differences in fat percentage.

Selection for high growth in beef cattle did not result in substantial changes in body composition. Carcass backfat was higher in steers from the high growth line compared to the low growth line. However, kidney, pelvic, and heart fat percentage, loin muscle area, and marbling score were not different between high and low growth lines (Castro Bulle et al., 2007).

A single generation of selection for RFI in beef cattle resulted in significant changes in body composition traits. Total dissected carcass fat (intramuscular and subcutaneous fat combined) was greater in the high RFI line than the low line. However, individually intramuscular and subcutaneous fat content did not differ between lines. Correlations between EBVs for subcutaneous, intramuscular and non-carcass fat with sire RFI were positive, again indicating that high RFI animals, or less efficient animals, are correlated with increased fat content (Richardson et al., 2001). There was also a negative correlation between RFI and chemical protein content indicating that more efficient animals have a higher protein content (Richardson and Herd, 2004).

Selection for residual feed intake in pigs resulted in correlated changes in body composition as well. Notably, both of these selection experiments accounted for backfat in their calculations of RFI, which would minimize any correlated response in fat content when selecting for more efficient animals. The low RFI line developed by Cai et al. (2008) had 1.99 mm less backfat and 1.05% less intramuscular fat, indicating that improved efficiency also reduced fat content. Gilbert et al. (2007) developed divergent lines for high and low RFI which also showed differences in fat content traits. There was

a positive phenotypic correlation between RFI and carcass backfat thickness, indicating that reducing RFI and improving feed efficiency will also reduce backfat. Genetic correlations between RFI and backfat traits were also positive, while genetic correlations with loin muscle area were negative.

Correlated responses in body composition due to selection for various measures of feed efficiency are extremely inconsistent. In some instances, there are no changes in fat versus protein content, but there is also evidence that increasing growth results in more fat gain. Selection that increases feed intake also seems associated with increased fat content. RFI selection does not seem to affect fat content, however backfat can be accounted for when calculating RFI and limit such correlated responses.

Reproductive traits

Sutherland et al. (1970) selected mice for gain for 9 generations and then employed further selection for 12 generations for gain, feed intake, or feed efficiency. Weaning weight was shown to decrease in all lines, though at a slower rate in the line selected only for gain across all 21 generations.

In rats selected for rapid lean growth, there were no significant differences in number born alive. When rats were selected for efficiency of lean growth, there was an initial tendency for lower numbers born alive after 5 generations of selection compared to a control line. However, after 14 generations of selection, differences in number born alive were insignificant. The efficiency line also had a smaller increase in weaning weight compared to the growth line (Notter et al., 1976; Wang and Dickerson, 1984).

Lines of mice selected for various measures of feed efficiency also showed differences in reproductive traits. Mice were selected for gain adjusted to constant intake, intake adjusted to a constant gain, intake adjusted for both gain and weight maintained, and the ratio of gain to feed. All of these lines showed reduced numbers born alive and numbers weaned compared to the control line, indicating that improved feed efficiency may result in poorer reproductive performance. However, the gain to feed line and the adjusted gain line had greater weaning weights, showing the productivity may be improved with improved feed efficiency (Holder et al., 1999).

In pigs divergently selected for RFI, there were weak genetic correlations between RFI and litter characteristics. Average piglet body weight at 21 d had a negative genetic correlation of -0.27 with RFI, indicating the lower RFI line was associated with greater piglet growth. However, the genetic correlation between the sow's RFI measurement was high and positive with average piglet body weight, demonstrating that the sow consumes more feed during lactation and diverts that feed toward litter growth. Additionally, the low RFI line produced approximately 0.5 additional piglets at birth, 21 d, and weaning than the high line and had greater litter growth after 6 generations of selection. However, in lactating sows, the low RFI line lost more body weight and back fat than sows from the high RFI line, which could hinder long term reproductive performance (Gilbert et al., 2012).

Correlated responses in reproductive performance are also variable. Selecting for increase growth seems to divert energy to gain, and negatively affect litter size, though in most cases the reduction is small. Selection for RFI does not seem to affect litter size and performance, suggesting that RFI is independent of reproductive performance.

Divergent selection for heat loss in mouse lines

Direct response in heat loss

In order to study the potential for reducing maintenance energy requirements by selection, Nielsen et al. (1997b) created divergent lines of mice selected for heat loss measured via direct calorimetry. A high heat loss (MH) line and low heat loss (ML) line were divergently selected in 3 independent replicates, along with an unselected control line (MC), resulting in 9 independent lines. The MH line was considered to have a high maintenance energy requirement, the ML line a low maintenance energy requirement, with the MC line being intermediate. Selection occurred for 16 generations then was relaxed for 26 generations before being renewed for 9 generations. Figure 1.1 illustrates the divergence achieved in heat production through selection.

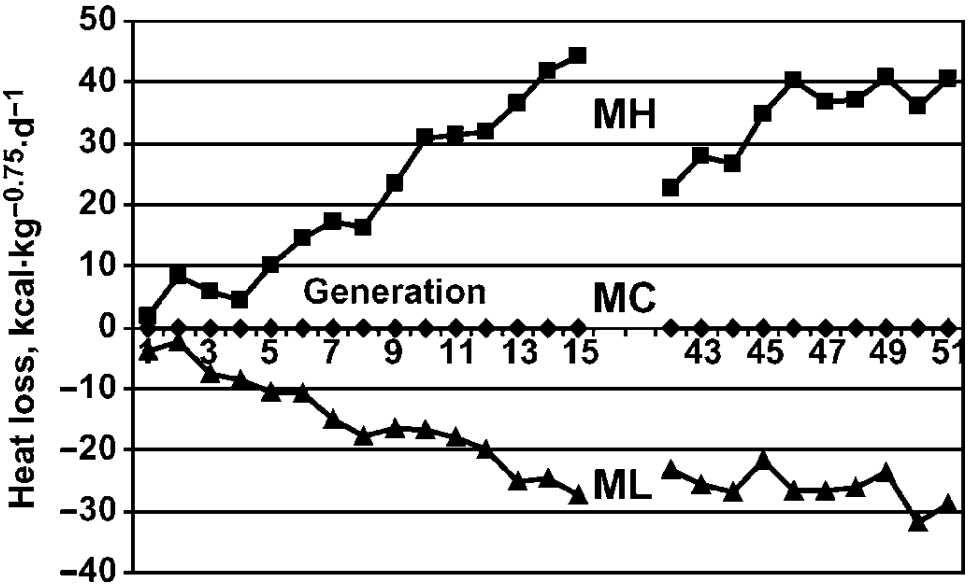

Figure 1.1: Line (MH: high heat loss; ML: low heat loss; MC: unselected control, set to zero) means for heat loss across 3 replicates for each line (McDonald et al., 2007).

The difference in heat loss between MH and ML lines averaged 53.6% as a percentage of the MC mean after the initial 15 generations of selection and was reduced to 34.4% after the period of relaxed selection. After selection was resumed, divergence was 55.7%.

Average change in heat loss per generation was 4.70 ± 0.09 kcal/kg$^{0.75}$/d during the original selection and 2.49 ± 0.30 kcal/kg$^{0.75}$/d during the second period of selection. Heritability, determined by regression of divergence of response on the difference in realized cumulative selection differentials between MH and ML, averaged 0.28 ± 0.003 for the first 15 generations of selection and 0.14 ± 0.01 in the last 9 generations of selection (McDonald and Nielsen, 2007; Nielsen et al., 1997b).

Correlated response in feed intake and efficiency

A correlated response in feed intake per unit of body weight was consistently observed in several studies with these mouse lines. After the initial 15 generations of selection, the difference between MH and ML averages was 20.6% relative to the MC average (Nielsen et al., 1997a). The genetic correlation between heat loss and feed intake was estimated to be between 0.27 and 0.40. The divergence in feed intake was maintained at 21.5% after the period of relaxed selection. After renewed selection, the difference in feed intake between MH and ML had increased to 34.0% of the MC mean. This difference in feed intake has been observed in several subsequent studies utilizing these lines (McDonald and Nielsen, 2007; Moody et al., 1997; Murphy et al., 2013). Additionally, there was no divergence in body weight between MH and ML mice and any point in the selection program, though both selection lines weighed less than the unselected MC line (McDonald and Nielsen, 2007; Murphy et al., 2013; Nielsen et al., 1997a). Therefore, ML mice were determined to have a more desirable feed efficiency. Eggert and Nielsen (2006) used several regression methods to separate feed intake into components for maintenance and growth. Growth coefficients were variable across lines,

but MH mice had a 16% higher maintenance energy requirement than ML mice. This confirms that reduction in feed intake in these lines is due to lower maintenance energy requirements.

Correlated response in body composition

Nielsen et al. (1997a) found that ML mice, at 16.9% fat, were significantly fatter than MH mice (16.0%) after 14 generations of selection, while MC mice were intermediate (16.4%). Kgwatalala and Nielsen (2004) found a slightly larger difference in fat percentage, with ML at 16.32% and MH at 14.45%, in mice subjected to different environmental temperatures. Moody et al. (1997) also demonstrated that MH mice had 40% less fat than ML mice. MH mice also had significantly larger livers and hearts than ML mice expressed as a percentage of total body weight. However, liver weight was not consistently larger in subsequent studies (Murphy et al., 2013). Additionally, Leamy et al. (2005) found liver weight to have a low genetic correlation with heat loss (0.06). Therefore, ML mice may have an undesirable body composition compared to MH mice.

Correlated response in reproductive performance

Nielsen et al. (1997a) and McDonald and Nielsen (2007) both found a significant difference in litter size, with ML mice having smaller litters than MH and MC mice intermediate. Nielsen et al. (1997a) determined that this was due to differences in ovulation rate. Additionally, ML litters tended to weigh less than MH litters, largely due to significantly lower milk production in ML dams (McDonald and Nielsen, 2006). However, McDonald and Nielsen (2007) showed that ML mice tended to have a higher

conception rate than MH mice. Overall, ML mice appear to have reduced reproductive performance when compared to MH mice.

Lifecycle efficiency

Analyzing the effects of selection on lifetime biological efficiency is a useful approach to determine the effect of changing maintenance energy requirements, considering the potential for negative correlated responses in economically important traits such as body composition and reproductive performance. Lifetime efficiency is modeled by establishing a measure of output and input relevant to livestock production. Output could be weaning weight or weight at slaughter (on a total or lean basis), which would be affected by changes in growth, body composition, and reproductive rates. Input is primarily feed intake, as it represents the largest cost, but other costs related to management can be factored into the model as well. Previous studies have calculated lifetime efficiency in several species and analyzed which inputs and outputs have an impact on efficiency.

Mice

Newman et al. (1985a) used mice to define a bioeconomic objective in mice and partition this objective into inputs and outputs to analyze lifetime efficiency. Inputs included feed for parents and offspring, labor, facilities, and costs of breeding stock. Outputs were lean yield produced by offspring and culled parents. Data came from a year-long experiment where multiple lines of mice were continuously cohabitated and allowed to reproduce. Feed intake, reproductive performance, growth, and body

composition were measured throughout the entire year on selected offspring and mating pairs. Progeny were kept for either a maximum of 42 or 49 d post-weaning to compare alternate slaughter ages. Culling of parents was based on age, number of litters produced, or interval between litters and different termination criteria were evaluated to identify which were most economically efficient. Results showed that the extra 7 d of feed intake for offspring culled at 49 d instead of 42 d was not offset by the increased final carcass size, particularly since older mice had less proportionate lean. Otherwise, reproductive performance had the largest impact on economic efficiency. Lines that produced more offspring, either due to larger litter sizes or greater reproductive longevity, had both a greater output, but required larger inputs as well. However, improved reproductive output lowers costs on per weaned animal since total costs are distributed over more animals (Newman et al., 1985b).

Swine

Tess et al. (1983b) used a deterministic model to estimate life cycle biological and economic efficiency of pigs in terms of total weight production or lean production when maintained to a common, predetermined genetic level of performance. Metabolizable energy intake was estimated by calculating the energy required for maintenance and a desirable rate of growth for gilts, energy for maintenance and gestation or lactation for sows, and energy for maintenance and growth for post-weaning offspring using NRC equations. Offspring growth was simulated using equations from the literature, and litter size and weight were estimated from previous data, as well as carcass size and composition of offspring. Once the model was finalized, percent fat, growth rate, age at

puberty, number born alive, pre-weaning viability, and conception rate were varied to determine the direction and magnitude of the effect of these traits on efficiency (Tess et al., 1983a). Faster growth rate increased sow energy requirements, but this was offset by lower energy requirements in growing animals due to these animals requiring fewer days on feed. Leaner pigs had an improved efficiency despite a higher maintenance energy requirement. Decreasing age at puberty and increasing conception rate improve efficiency as less energy is required for replacement gilts. Increasing number born alive did not excessively increase energy intake and distributes costs over more animals, thereby improving efficiency. Improved pre-weaning viability also greatly improved efficiency. Conception rate, number born alive, and viability were most important for improving economic efficiency and biological efficiency of total weight production, while growth rate and percent fat were most important for biological efficiency of lean production.

Beef cattle

Multiple studies have attempted to model lifecycle efficiency of beef production systems. Davis et al. (1983a) measured lifetime feed intake of cows through 3 lactations including progeny creep feed intake and feed intake measured on progeny from 60 to 240 d of age. Dams were weighed at weaning of each parity and offspring weaning weight was recorded. The expected number of subsequent parities and weaning rates of said parities were calculated assuming a 10% attrition rate to calculate an age distribution of the herd at equilibrium. Efficiency was then evaluated using multiple measures: one divided total weight of all progeny born and salvage weight of the dams (discounted by

4/7) by total feed intake of both progeny and dams, and the other only included progeny weight in the numerator. Breeding cows at a younger age and increasing number of progeny weaned improved efficiency rates since breeding at a younger age reduces days on feed before first parity. Feed intake of dams was found to be the most important factor affecting feed intake, accounting for 90% of all feed inputs. Feed intake of offspring had a much smaller effect due to the fact that it accounts for a much smaller proportion of total lifecycle. Smaller dams had lower maintenance requirements resulting in lower feed intake and improved feed efficiency. Weaning weight had a positive, but small effect on cow efficiency. Cows with a higher fat percentage were found to be less efficient due to cows becoming fat without conceiving (Davis et al., 1983b).

Davis et al. (1994a) simulated inputs and outputs for heifers from date of purchase to slaughter in a model that was equivalent to simulating a single year of a herd at equilibrium. Inputs consisted of metabolizable energy intake for maintenance and gain, calculated from NRC equations for both dams and calves to weaning. Output measures included cow weight and calf weight, with a discount placed on culled cows. Much of the focus of this study was evaluating breed differences and the effect of maternal heterosis on biological efficiency. Crossbred dams had higher reproductive rates, produced more calves that were also larger, and required fewer replacements, so they had much higher outputs. However, crossbred cows are also larger and stay in the herd longer, therefore their inputs are also larger. Per unit of output, input costs were reduced by 15% when utilizing crossbred dams, so ultimately the improved reproductive output was beneficial and overcomes increased inputs (Davis et al., 1994b).

Naazie et al. (1997; 1999) developed a model to evaluate lifecycle efficiency in beef cattle herds of various breeds. Total inputs included feed intake of offspring from birth to slaughter and feed intake of breeding bulls and dams predicted using NRC equations. Total output was the lean output of offspring at slaughter and culled dams and bulls computed from field data. Lifecycle efficiency was more improved in slower maturing animals despite the increased energy intake required for their offspring because slower maturing dams will have smaller maintenance energy requirement and therefore consume less feed than faster maturing dams. Since the intake required for dams is the largest input, this outweighs improvements on the offspring side. Improved reproductive rate was also extremely important in improving lifecycle efficiency, so long as achieving such improvement does not require excessive costs. Lifecycle efficiency declines as cows are maintained longer, until 6 yr of age, where some breeds actually showed a recovery in efficiency. The decline is likely due to increased intake of dams without any associated increase in output.

Montaño-Bermudez and Nielsen (1990) estimated the biological efficiency of beef cattle with different genetic potential for milk, separating cows into a low, medium, and high group. Authors used survival probabilities and Markov-chain methods to calculated the age distribution of the herd at equilibrium and weighted inputs and outputs by the number of cows in the herd at each stage (Azzam et al., 1990). Outputs were slaughter weights of steers and heifers. Inputs were energy intake including energy for maintenance, gain, and gestation in the case of the cow herd. Energy for maintenance was calculated as the sum of metabolic body weights over the feeding period multiplied by the maintenance energy requirements, determined in a previous study (Montano-

Bermudez et al., 1990). The low milking group had the greatest biological efficiency and the high group was the least efficient. Maintenance energy was responsible for 64 to 67% of the total energy requirements and both the medium and high groups had a higher maintenance energy requirement than the low milking group. Calves from the high and medium group also had a higher maintenance energy requirement than calves from the low group. Overall, cows with low milking potential have a lower maintenance energy requirement and therefore and improved biological efficiency (Montano-Bermudez and Nielsen, 1990).

Results of modeling lifecycle efficiency provide indications of which factors have the largest effect on improving an animal's overall efficiency throughout its lifetime. Feed intake to maintain reproductive parents was often the largest input, particularly in beef cattle systems. Maintenance energy requirements usually represented the largest component of total energy intake and therefore had the largest effect on overall efficiency. Improving growth is also important to reducing inputs as faster growing animals have fewer days on feed. Reproductive performance had the largest effect on output performance, especially in litter bearing species. Increasing reproductive longevity was particularly important, even though it increases input costs to maintain the dams, those costs are distributed over more offspring. Overall, improving lifecycle feed efficiency requires reducing feed intake, particularly for maintenance energy requirements of reproductive animals, while improving or at least not negatively affecting growth or reproductive performance. Such improvement could potentially be gained through selection.

Effect of selection for feed efficiency

Wang and Dickerson (1984) analyzed net lifecycle efficiency of rats selected for rate or efficiency of lean growth, by simulating a production system where rats were culled after producing a single litter. Efficiency was measured as the ratio of total inputs (feed and non-feed) to protein output. Inputs and outputs were calculated separately for different life stages including breeding, gestation, lactation, and growth. Offspring from the selection lines produced 12 to 20% more protein output, however they also consumed 7 to 18% more feed than a control line. Females in the rapid growth line consumed more feed during gestation and lactation, while the efficiency line was similar to controls. When inputs and outputs were summed across all life stages, both selection lines had an improved overall efficiency compared to the control. Overall, the rapid growth line had the best lifecycle efficiency due to its faster growth rate as well as a lower metabolic rate and slightly better reproductive performance. Poorer reproductive performance in the efficiency line limited its lifecycle efficiency compared to the fast growth line, even though this line had a lower maintenance cost. However, authors noted that reduce reproductive performance has less of an impact on lifecycle efficiency in litter-bearing species. In general, authors demonstrated that selection for improved efficiency in growing offspring could improve lifecycle efficiency as well.

Summary

Improving feed efficiency is a primary goal of the livestock industry, with producers seeking improvement through a variety of methods. Feed efficiency is usually measured as a ratio of outputs to inputs, therefore improvement can be achieved by

focusing on either side. Historically, more emphasis was placed on outputs and improving growth rates, but more recently, focus has shifted to changing inputs (primarily feed intake or ME intake). Metabolizable energy intake from feed is partitioned into multiple, independent components. Maintenance energy requirements represent the largest component, accounting for up to 70 to 75% of energy intake in some species, and theoretically, changing maintenance energy requirements would not affect growth. Therefore, altering maintenance energy requirements could be a beneficial avenue to improve feed efficiency.

Feed efficiency can be improved utilizing multiple methods, but selection is the primary path from a genetics standpoint. In order for selection to be effective, the trait of interest must contain genetic variation, and sufficient genetic variation in maintenance energy requirements has been shown. There is, in fact, more variation in maintenance energy requirements than production energy requirements. However, maintenance energy requirements are difficult to measure, so initial selection programs selected for improved growth rates, feed efficiency ratios, or residual feed intake. Additionally, heat production was introduced as an indicator trait for maintenance energy requirements.

Selection for increased growth rate has been highly effective, however it is associated with an increase in feed intake. Generally, the improvement in gain outweighs the increase in feed intake, resulting in improved feed efficiency, but leaves potential for a more accurate and efficient method. Selecting directly for feed efficiency has not been particularly effective as it is a lowly heritable trait and ratios often present difficulties as selection criteria. Selection for residual feed intake has been shown to be effective at reducing feed intake independent of growth, and therefore improves feed efficiency in

most livestock species. This result is presumed to be primarily the result of reducing feed intake for maintenance energy requirements, based on the way residual feed intake is calculated.

Selection to improve feed efficiency has been associated with reduced maintenance energy requirements in most livestock species and in mice. Residual feed intake, in particular is associated with lower maintenance energy requirements and lower fasting heat production. Results from a long-term selection program for heat production (as an indicator of maintenance energy requirements) in mice have shown substantial divergence in heat production, and presumably maintenance energy requirements. Significant reduction in feed intake has occurred, with no affect on final body weight, therefore demonstrating an improvement in feed efficiency in this selection experiment.

Selection to improve feed efficiency, regardless of how it is measured, is negatively correlated with some economically important traits. Correlated response in body composition seems to vary depending on species and selection trait. Selection for increased growth increased fat content in some instances, particularly if selection resulted in increased feed intake. Selecting for residual feed intake seldom affected fat content, as it is generally accounted for when calculating residual feed intake. Correlated response in reproductive performance was also variable. When selection resulted in decreased feed intake, energy was sometimes diverted to maintenance or growth resulting in a slight decrease in litter size. Again, selection for residual feed intake did not affect reproductive performance, indicating that maintenance energy does not affect reproduction.

However, there is evidence that low maintenance animals have a higher fat percentage compared to high maintenance animals. Additionally, low maintenance animals were shown to have smaller litter sizes and smaller weaning weights, due to poorer milk production. These two negative responses could negate some of the benefit of reduced feed intake in lower maintenance animals. Survival rates of low maintenance animals compared to high maintenance animals should also be considered. Finally, reducing maintenance energy requirements should be viewed in terms of lifecycle production, to observe how conflicting selection responses balance out over an entire lifetime.

Lifecycle efficiency can be calculated through deterministic or simulation models that account for inputs and outputs required to sustain a livestock production system. Such models can help elucidate which factors have a larger effect on the efficiency of system as a whole, rather than at a single life stage. Inputs largely consist of feed intake, though management costs can be included, and output is usually final carcass weight, which can be adjusted to only include lean product. Results from multiple studies indicate that reduced maintenance energy requirements are of major importance to improving lifecycle feed efficiency on the input side of the model. Reproductive performance and growth rate are output factors with a large effect on lifecycle efficiency. Reproductive longevity was also found to be important for improving lifecycle efficiency. In rats, selection for improved growth rate and efficiency of growth were associated with an overall improvement in lifecycle efficiency.

In the mouse lines divergently selected for heat loss, a lifecycle efficiency evaluation would be useful to determine the benefit of selection for reduced maintenance

energy requirements. Low maintenance animals consume less feed, and should therefore have lower overall inputs than high maintenance animals. However, there is some evidence that they have poorer reproductive performance and body composition, which could negatively affect outputs. Additionally, the longevity of low maintenance animals under livestock production culling structures is unknown, and a decrease in reproductive longevity in one line could affect lifecycle efficiency. Evaluating these lines in a lifecycle efficiency manner will help clarify the benefit of selecting for reducing maintenance energy requirements.

Literature cited

Archer, J. A., and W. S. Pitchford. 1996. Phenotypic variation in residual food intake of mice at different ages and its relationship with efficiency of growth, maintenance and body composition. Anim. Sci. 63: 149-157.

Azzam, S. M., A. M. Azzam, M. K. Nielsen, and J. E. Kinder. 1990. Markov chains as a shortcut method to estimate age distributions in herds of beef cattle under different culling strategies. J. Anim. Sci. 68: 5-14.

Barea, R., S. Dubois, H. Gilbert, P. Sellier, J. van Milgen, and J. Noblet. 2010. Energy utilization in pigs selected for high and low residual feed intake. J. Anim. Sci. 88: 2062-2072.

Biondini, P. E., T. M. Sutherland, and L. H. Haverland. 1968. Body composition of mice selected for rapid growth rate. J. Anim. Sci. 27: 5-12.

Byerly, T.C. 1941. Feed and other costs of producing market eggs. Bull. A1. (Technical). Univ. Maryland Agric. Exp. Stn., College Park, MD.

Cai, W., D. S. Casey, and J. C. M. Dekkers. 2008. Selection response and genetic parameters for residual feed intake in yorkshire swine. J. Anim. Sci.. 86: 287-298.

Castro Bulle, F. C. P., P. V. Paulino, A. C. Sanches, and R. D. Sainz. 2007. Growth, carcass quality, and protein and energy metabolism in beef cattle with different growth potentials and residual feed intakes. J. Anim. Sci. 85: 928-936.

Davis, K. C., M. W. Tess, D. D. Kress, D. E. Doornbos, and D. C. Anderson. 1994a. Life cycle evaluation of five biological types of beef cattle in a cow-calf range production system: I. Model development. J. Anim. Sci. 72: 2585-2590.

Davis, K. C., M. W. Tess, D. D. Kress, D. E. Doornbos, and D. C. Anderson. 1994b. Life cycle evaluation of five biological types of beef cattle in a cow-calf range production system: Ii. Biological and economic performance. J. Anim. Sci. 72: 2591-2598.

Davis, M. E., J. J. Rutledge, L. V. Cundiff, and E. R. Hauser. 1983a. Life cycle efficiency of beef production: I. Cow efficiency ratios for progeny weaned. J. Anim. Sci. 57: 832-851.

Davis, M. E., J. J. Rutledge, L. V. Cundiff, and E. R. Hauser. 1983b. Life cycle efficiency of beef production: II. Relationship of cow efficiency ratios to traits of the dam and progeny weaned. J. Anim. Sci. 57: 852-866.

Derno, M., W. Jentsch, M. Schweigel, S. Kuhla, C. C. Metges, and H.-D. Matthes. 2005. Measurements of heat production for estimation of maintenance energy requirements of hereford steers. J. Anim. Sci. 83: 2590-2597.

Eggert, D. L., and M. K. Nielsen. 2006. Comparison of feed energy costs of maintenance, lean deposition, and fat deposition in three lines of mice selected for heat loss. J. Anim. Sci. 84: 276-282.

Ferrell, C. L., and T. G. Jenkins. 1985. Cow type and the nutritional environment: Nutritional aspects. J. Anim. Sci. 61: 725-741.

Gabarrou, J. F., P. A. Geraert, N. Francois, S. Guillaumin, M. Picard, and A. Bordas. 1998. Energy balance of laying hens selected on residual food consumption. Br. Poult. Sci. 39: 79-89.

Gilbert, H., J.-P. Bidanel, Y. Billon, H. Lagant, P. Guillouet, P. Sellier, J. Noblet, and S. Hermesch. 2012. Correlated responses in sow appetite, residual feed intake, body

composition, and reproduction after divergent selection for residual feed intake in the growing pig. J. Anim. Sci. 90: 1097-1108.

Gilbert, H., J. P. Bidanel, J. Gruand, J. C. Caritez, Y. Billon, P. Guillouet, H. Lagant, J. Noblet, and P. Sellier. 2007. Genetic parameters for residual feed intake in growing pigs, with emphasis on genetic relationships with carcass and meat quality traits. J. Anim. Sci. 85: 3182-3188.

Gilbert, H., J. P. Bidanel, J. Gruand, J. C. Caritez, Y. Billon, P. Guillouet, J. Noblet, and P. Sellier. 2006. Genetic parameters and responses to divergent selection for residual feed intake in the growing pig. Proc. 8th World Congr. Genet. Appl. Livest. Prod., Belo Horizonte, Brazil.

Hastings, I. M., S. M. Moruppa, L. Bünger, and W. G. Hill. 1997. Effects of selection on food intake in the adult mouse. J. Anim. Breed. Genet. 114: 419-434.

Herd, R. M., and P. F. Arthur. 2009. Physiological basis for residual feed intake. J. Anim. Sci. 87: E64-71.

Herd, R. M., V. H. Oddy, and E. C. Richardson. 2004. Biological basis for variation in residual feed intake in beef cattle 1. Review of potential mechanisms. Aust. J. Exp. Agr. 44: 423-430.

Holder, R. B., A. S. Moura, and W. R. Lamberson. 1999. Direct and correlated responses to selection for efficiency of lean gain in mice. J. Anim. Sci. 77: 575-581.

Hotovy, S. K., K. A. Johnson, D. E. Johnson, G. E. Carstens, R. M. Bourdon, and G. E. Seidel. 1991. Variation among twin beef cattle in maintenance energy requirements. J. Anim. Sci. 69: 940-946.

Johnston, S. L., D. M. Souter, S. S. Erwin, B. J. Tolkamp, J. M. Yearsley, I. J. Gordon, A. W. Illius, I. Kyriazakis, and J. R. Speakman. 2007. Associations between basal metabolic rate and reproductive performance in c57bl/6j mice. J. Exp. Biol. 210: 65-74.

Jungst, S. B., L. L. Christian, and D. L. Kuhlers. 1981. Response to selection for feed efficiency in individually fed yorkshire boars. J. Anim. Sci. 53: 323-331.

Kgwatalala, P. M., and M. K. Nielsen. 2004. Performance of mouse lines divergently selected for heat loss when exposed to different environmental temperatures. II. Feed intake, growth, fatness, and body organs. J. Anim. Sci. 82: 2884-2891.

Klein, M., R. Schadereit, H. Scholze, and U. Renne. 1999. Studies on energy metabolism in lines of mice selected for different growth parameters. J. Anim. Physiol. An. N. 81: 75.

Koch, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. J. Anim. Sci. 22: 486-494.

Leamy, L., K. Elo, M. Nielsen, L. D. Van Vleck, and D. Pomp. 2005. Genetic variance and covariance patterns for body weight and energy balance characters in an advanced intercross population of mice. Genet. Sel. Evol. 37: 151-173.

Luiting, P., J. W. Schrama, W. van der Hel, and E. M. Urff. 1991. Metabolic differences between white leghorns selected for high and low residual food consumption. Br. Poult. Sci. 32: 763-782.

McDonald, J. M., and M. K. Nielsen. 2006. Correlated responses in maternal performance following divergent selection for heat loss in mice. J. Anim. Sci. 84: 300-304.

McDonald, J. M., and M. K. Nielsen. 2007. Renewed selection for heat loss in mice: Direct responses and correlated responses in feed intake, body weight, litter size, and conception rate. J. Anim. Sci. 85: 658-666.

Montano-Bermudez, M., and M. K. Nielsen. 1990. Biological efficiency to weaning and to slaughter of crossbred beef cattle with different genetic potential for milk. J.Anim. Sci. 68: 2297-2309.

Montano-Bermudez, M., M. K. Nielsen, and G. H. Deutscher. 1990. Energy requirements for maintenance of crossbred beef cattle with different genetic potential for milk. J. Anim. Sci. 68: 2279-2288.

Moody, D. E., D. Pomp, and M. K. Nielsen. 1997. Variability in metabolic rate, feed intake and fatness among selection and inbred lines of mice. Genet. Res. 70: 225-235.

Murphy, T. W., J. M. McDonald, and M. K. Nielsen. 2013. Hepatic mitochondrial efficiency in lines of mice differing in feed intake. J. Anim. Sci. 91: 2077-2082.

Naazie, A., M. Makarechian, and R. J. Hudson. 1997. Efficiency of beef production systems: Description and preliminary evaluation of a model. Agr. Sys. 54: 357-380.

Naazie, A., M. Makarechian, and R. J. Hudson. 1999. Evaluation of life-cycle herd efficiency in cow-calf systems of beef production. J. Anim. Sci. 77: 1-11.

Newman, S., D. L. Harris, and D. P. Doolittle. 1985a. Economic efficiency of lean tissue production through crossbreeding: Systems modeling with mice. I. Definition of the bioeconomic objective. J. Anim. Sci. 60: 385-394.

Newman, S., D. L. Harris, and D. P. Doolittle. 1985b. Economic efficiency of lean tissue production through crossbreeding: Systems modeling with mice. II. Reproduction-growth termination alternatives. J. Anim. Sci. 60: 395-412.

Nielsen, M. K., B. A. Freking, L. D. Jones, S. M. Nelson, T. L. Vorderstrasse, and B. A. Hussey. 1997a. Divergent selection for heat loss in mice: II. Correlated responses in feed intake, body mass, body composition, and number born through fifteen generations. J. Anim. Sci. 75: 1469-1476.

Nielsen, M. K., L. D. Jones, B. A. Freking, and J. A. DeShazer. 1997b. Divergent selection for heat loss in mice: I. Selection applied and direct response through fifteen generations. J. Anim. Sci. 75: 1461-1468.

Nkrumah, J. D., E. K. Okine, G. W. Mathison, K. Schmid, C. Li, J. A. Basarab, M. A. Price, Z. Wang, and S. S. Moore. 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methande production, and energy partitioning in beef cattle. J. Anim. Sci. 84: 145-153.

Noblet, J., X. S. Shi, and S. Dubois. 1993. Metabolic utilization of dietary energy and nutrients for maintenance energy requirements in sows: Basis for a net energy system. Brit. J. Nutr, 70: 407-419.

Notter, D. R., G. E. Dickerson, and J. A. Deshazer. 1976. Selection for rate and efficiency of lean gain in the rat. Genetics 84: 125-144.

Richardson, E. C., and R. M. Herd. 2004. Biological basis for variation in residual feed intake in beef cattle 2. Synthesis of results following divergent selection. Aust. J. Exp. Agr. 44: 431-440.

Richardson, E. C., R. M. Herd, V. H. Oddy, J. M. Thompson, J. A. Archer, and P. F. Arthur. 2001. Body composition and implication for heat production of angus steer progeny of parents selected for and against residual feed intake. Aust. J. Exp. Agr. 41: 1065-1072.

Selman, C., S. Lumsden, L. Bunger, W. G. Hill, and J. R. Speakman. 2001. Resting metabolic rate and morphology in mice (mus musculus) selected for high and low food intake. J. Exp. Biol. 204: 777-784.

Skinner-Noble, D. O., and R. G. Teeter. 2003. Components of feed efficiency in broiler breeding stock: Energetics, performance, carcass composition, metabolims, and body temperature. J. Poult. Sci. 82: 1080-1090.

Sutherland, T. M., P. E. Biondini, L. H. Haverland, D. Pettus, and W. B. Owen. 1970. Selection for rate of gain, appetite and efficiency of feed utilization in mice. J. Anim. Sci. 31: 1049-1057.

Tess, M. W., G. L. Bennett, and G. E. Dickerson. 1983a. Simulation of genetic changes in life cycle efficiency of pork production, II. Effects of components on efficiency. J. Anim. Sci. 56: 354-368.

Tess, M. W., G. L. Bennett, and G. E. Dickerson. 1983b. Simulation of genetic changes in life cycle efficiency of pork production. I. A bioeconomic model. J. Anim. Sci. 56: 336-353.

Wang, C.-T., and G. E. Dickerson. 1984. Selection for rate and for efficiency of lean growth in rats: Net life-cycle efficiency. J. Anim. Sci. 59: 49-66.

Williams, C. B., and T. G. Jenkins. 2003. A dynamic model of metabolizable energy utilization in growing and mature cattle. I. Metabolizable energy utilization for maintenance and support metabolism. J. Anim. Sci. 81: 1371-1381.

Chapter II

Abstract

Divergent selection for heat loss was implemented in mice creating high (MH) and low (ML) maintenance lines, and unselected control (MC) in 3 independent replicates. Mice from the ML line have improved feed efficiency, due to decreased maintenance energy requirement, but there is potential for a correlated decline in reproductive performance and survivability. Number fully formed (NFF), number born alive (NBA), number weaned (NW), litter weaning weight (LWW), pup weaning weight (PWW), fraction alive at birth (FAB), fraction alive at weaning (FAW), and birth interval were recorded at every parity on 21 mating pairs from each line × replicate combination cohabitated at 7 wk of age and maintained for up to 1 yr. Traits were summed over parities to evaluate lifetime production. Pairs were culled due to death or illness, no first parity by 42 d cohabitation, 2 consecutive litters with none born alive, 3 consecutive litters with none weaned, 42 d between parities, or average size of most recent 2 litters less than half the average of first 3 litters. Survival probabilities were produced and evaluated for each line and used to calculate mean number of parities using a Markov-chain algorithm assuming a maximum of 4, 6, 8, 10, or 12 parities or 1 yr. Line was insignificant for all litter traits, while NFF, NW, and FAB decreased with parity ($P < 0.05$) and PWW tended to increase ($P < 0.07$). MC mice had higher lifetime NW, LWW, and PWW ($P < 0.04$). Birth interval showed that MH mice had increasingly larger intervals, while remaining the same in ML mice ($P < 0.01$). In the survival analysis, MC mice had the greatest survival rates overall, but ML mice had the greatest rates in the period up to 5 parities, while MH mice had the greatest rates in later parities. This

resulted in greater mean number of parities for ML mice up to maximum of 8 parities and higher means for MH mice when the maximum number of allowed parities was 10 or higher. Reproductive performance was not substantially affected by changing maintenance energy requirements. The ML animals appear to survive well in early parities and produce more parities when a low number of maximum parities is enforced, but this benefit declines in later parities and MH animals survive better and increase mean number of parities when turnover rates are low. Therefore, selection for low maintenance animals may be beneficial for systems desiring a short generation interval, but less so for systems desiring longevity.

Introduction

Feed intake to meet maintenance energy requirements is the largest component of feed consumption and the largest economic input in livestock production systems (Ferrell and Jenkins, 1985; Noblet et al., 1993). Therefore, selection to reduce maintenance requirements without affecting output (reproduction, growth, etc.) could be a beneficial avenue to improve feed efficiency. Energy intake that is not stored as product is released as heat. Heat loss contains genetic variation and can be used as an indicator of maintenance energy requirements (Nielsen et al., 1997a,b; Williams and Jenkins, 2003).

Nielsen et al. (1997b) demonstrated that maintenance energy requirements could be altered by selection for heat loss measured by direct calorimetry. Selection over 16 generations in mice established a maintenance high (MH) and maintenance low line (ML), which differed significantly in heat loss, as well as feed intake per unit of body weight, with the ML line consuming less feed than the MH line (Nielsen et al., 1997a,b).

Selection was relaxed for 26 generations and then renewed for 9 generations, once again showing further response in heat loss and feed intake (McDonald and Nielsen, 2007). However, though ML mice tended to have higher conception rates, ML mice had smaller litter sizes (McDonald and Nielsen, 2007; Nielsen et al., 1997a). The ML mice also tended to have smaller litter weaning weights (LWW) due to poorer milk production (McDonald and Nielsen, 2006). Decline in productivity and reproductive performance may be a correlated response to selection for reduced maintenance energy requirements. Stayability may be reduced in ML mice if culling for poor reproductive performance is applied.

The objective of this study was to use these mouse lines to imitate a livestock system and test the hypothesis that reducing maintenance energy requirements negatively affects productivity, reproductive performance, and survivability, which could diminish the benefit in improved feed efficiency.

Materials and methods

Experimental animals

All animal procedures were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Animals used in this study were sampled from lines of mice divergently selected for heat loss, as an indicator of maintenance energy requirements (MH = maintenance high, ML = maintenance low, MC = unselected control) and have been previously described by Nielsen et al. (1997b). Briefly, heat loss per unit of metabolic body weight ($kcal \cdot kg^{-0.75} \cdot d^{-1}$) was measured on individual males 9 to 11 wk of age by placing them in direct calorimeters for 15 h overnight. Selection

occurred in 3 replicates, creating 9 independent lines. Initial selection lasted for 16 generations, then selection was relaxed for 26 generations, though independence of the lines was maintained. Selection was then resumed for 9 generations, based on the same selection criteria. In the present study, 21 mating pairs were selected from each of the 9 line × replicate combinations from generation 70, resulting in 189 total pairs of mice. Pairs were cohabitated at 7 wk of age and maintained together for the duration of the study, unless culled. Mice were housed in plastic cages with wire lids and had ad libitum access to water and feed (Teklad diet 2019: 19% crude protein, 9.0% crude fat, 2.6% crude fiber, and 3.3 kcal of ME/g; Harlan Teklad, Madison, WI). Rooms housing animals were subjected to a 12 h:12 h light:dark cycle and ambient temperature was maintained at 23.5 ± 1.0°C.

Measuring reproductive and maternal performance

Litter traits were recorded for each pair at every parity. Number fully formed (NFF) and number born alive (NBA) were recorded within 24 h after birth. Litters were weaned at 21 d after birth, when number weaned (NW) and litter weaning weight (LWW) were recorded. Pup weaning weight (PWW) was calculated as LWW/NW, fraction alive at birth (FAB) as NBA/NFF, and fraction alive at weaning (FAW) as NW/NBA. Each trait was also summed across parities for each pair to obtain lifetime performance measures. Birth interval was recorded as the number of days between consecutive parities and was recorded for all parity intervals.

Culling criteria

Several culling criteria designed to be similar to criteria used in livestock production systems were established. Culling criteria were purposely made more lenient to insure enough pairs would survive into later parities for accurate analysis. Pairs were culled due to death or illness of either member. If the male died, the female was maintained for 21 d (normal gestation length is 20 to 21 d in mice) to determine if she was pregnant. In the case of pregnancy, the female was allowed to deliver and wean her litter; if open, she was culled. Additionally, pairs were culled due to poor reproductive performance. If no first litter was produced 42 d (2 full gestations lengths) after cohabitation, the pair was considered reproductively unsound and culled. Pairs were also culled if they produced 2 consecutive litters with none born alive or 3 consecutive litters with none weaned. If the birth interval between consecutive parities was longer than 42 d, then the pair was culled. Litter size was deemed too small when the average of the most recent 2 litters was less than half the average of the first 3 and the pair was culled. Otherwise pairs were maintained for 1 yr. Culled animals were euthanized by CO_2 asphyxiation.

Linear models analysis

Traits were analyzed using the GLIMMIX procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC) with the following model:

$$y_{ijkl}=\mu+line_i+parity_j+line*parity_{ij}+rep_k+rep*line_{ik}+rep*parity_{jk}+rep*line*parity_{ijk}+e_{ijkl},$$

where y_{ijkl} is the phenotypic record for each recorded trait, $line_i$ is the fixed effect of line (MH, ML, or MC), $parity_j$ is the fixed effect of the parity when the trait was recorded (1 to 11), and rep_k is the random effect of replicate (1, 2, or 3), and e_{ijkl} is random error. Data for NW were analyzed both including and excluding records on parities where no pups were weaned. Parity was treated as a repeated measure with pair nested within rep × line as the subject. An autoregressive heterogeneous variance component structure was chosen based on Akaike information criterion, corrected for finite sample size (Burnham and Anderson, 2002).

The following model was used to analyze lifetime performance:

$$y_{ijk}=\mu+line_i+rep_j+rep*line_{ij}+e_{ijk},$$

where y_{ijk} is the phenotypic record for each summed trait, $line_i$ is the fixed effect of line (MH, ML, or MC) and rep_j is the random effect of replicate (1, 2, or 3). For both models, orthogonal contrasts were used to test for selection response (MH vs. ML) or asymmetry of response [(MH + ML)/2 vs. MC].

Birth interval was analyzed by linear regression with the following model:

$$y_{ijkl}=(\beta_0+rep_{0i}+line_{0k})+(\beta_1+rep_{1i}+line_{1k})*X_{ijk}+e_{ijkl},$$

where y_{ijkl} is the number of days between consecutive parities; β_0 and β_1 are the overall intercept and slope, respectively; rep_{0i} and rep_{1i} are the random effect of replicate on the intercept and slope, respectively; $line_{0k}$ and $line_{1k}$ are the effects of line on the intercept and slope, respectively; and X is the parity interval (parity 1 to 2, parity 2 to 3, etc.). Contrasts were used to test for a difference in slopes and intercepts between selection lines.

Survival analysis

Survival analysis was performed using the LIFETEST and PHREG procedures in SAS 9.3 (SAS Inst. Inc., Cary, NC). Survival was measured in maximum number of parities recorded for the pair before culling; thus time was treated as a discrete measure. All pairs were culled before the end of 1 yr of cohabitation, so censoring of the data was not necessary. Data were analyzed over the entire study and in two periods (Period 1: ≤ 5 parities, Period 2: > 5 parities). Survival functions were produced for each line using Kaplan-Meier estimates, defined as:

$$\hat{S}(t) = \prod_{i:t_i \leq t} \left(1 - \frac{d_i}{n_i}\right),$$

where $\hat{S}(t)$ is the Kaplan-Meier estimator of survival to time t, d_i is the number of individuals culled at time t_i (parity 1 to 12), and n_i is the total number of animals at risk of culling at time t_i (Allison, 1997). Log-rank tests were used to determine differences in survival functions between lines.

Hazard functions were produced for each line using a Cox discrete hazard model:

$$\log[h_i(t)] = \ln[h_0(t)] (\beta_1 x_{i1}),$$

where $h_0(t)$ is the baseline hazard at time t, β_1 is the coefficient associated with line (MH, ML, or MC) and x_{i1} is parity number. The function $h_i(t)$ is defined as $P_{it}/(1 - P_{it})$ where P_{it} is the conditional probability individual i is culled at time t, given that it has not already been culled (Allison, 1997). Log-rank tests of hazard ratios were used to compare lines.

A competitive risk analysis was performed to evaluate the risk of each culling criterion for all experimental animals and also within each line. Models were identical to the Cox discrete hazard model above with cause of culling incorporated as censoring. For

example, when assessing the risk of culling due to death or illness, animals that were culled for other reasons were treated as censored (Allison, 1997).

Parity equilibrium

Survival probabilities were used to estimate the parity distribution of a population of mice within each line, to compare mean number of parities produced by such populations and potential differences in replacement rates. This was achieved using Markov-chain methods described by Azzam et al. (1990). In short, a transition matrix (P) was created relating the probability a mating pair at a certain parity would be retained in the population for an additional parity, or if they would be replaced by a parity 1 mating pair. A column vector, π, was defined as the proportion of mating pairs in the population at each parity after the parity distribution has reached equilibrium, assuming population size is constant. Proportions were found by simultaneously solving the set of equations $\pi = P\pi$. However, this equation does not have a direct solution in its current form. This was corrected by arbitrarily eliminating one equation and replacing it with $\Sigma_i \pi_i = 1$. The set of equations can then be solved by Gaussian elimination. The average number of parities was then calculated by multiplying π by a vector of corresponding parities. Parity distributions were calculated assuming animals were maintained a maximum of 4, 6, 8, 10, or 12 parities, or for 1 yr as was done in the study. More details are available in Appendix A.

Results and discussion

Reproductive and maternal performance

Changes in litter size traits, across parities, are shown in Figure 2.1. Parity did have a significant effect on some litter size traits, with NFF and NW both significantly decreasing in later parities ($P < 0.01$). As shown in Figure 2.2, FAB also decreased in later parities ($P < 0.02$).

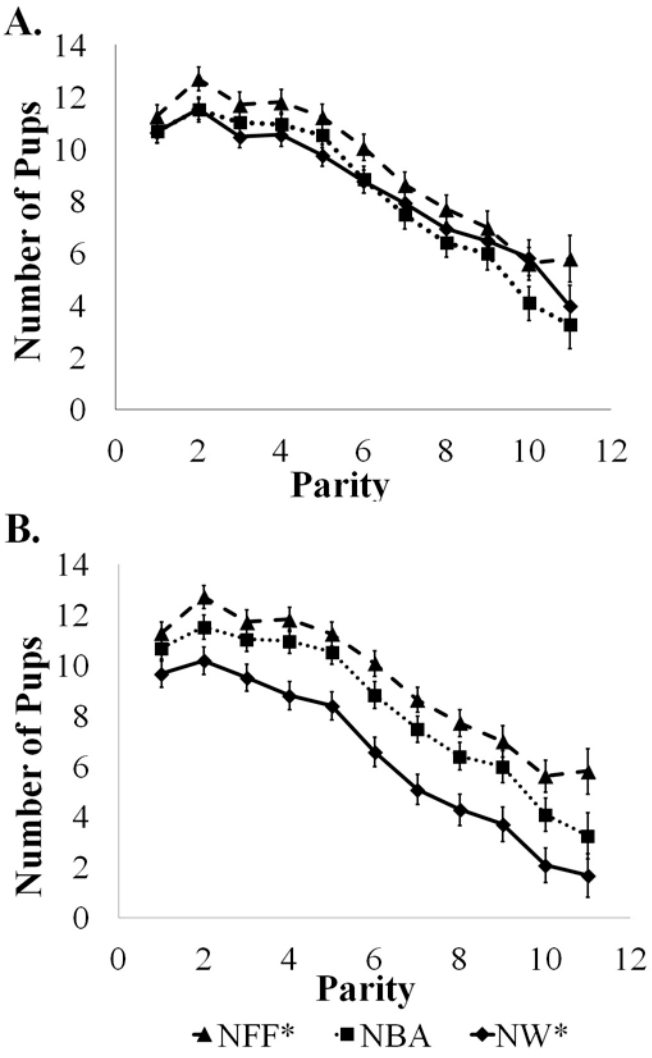

Figure 2.1. Number fully formed (NFF), number born alive (NBA), and number weaned (NW) by parities when litters with none weaned were excluded (Panel A) or included (Panel B) (* indicates significant effect of parity on trait ($P < 0.05$). Error bars denote SEM.

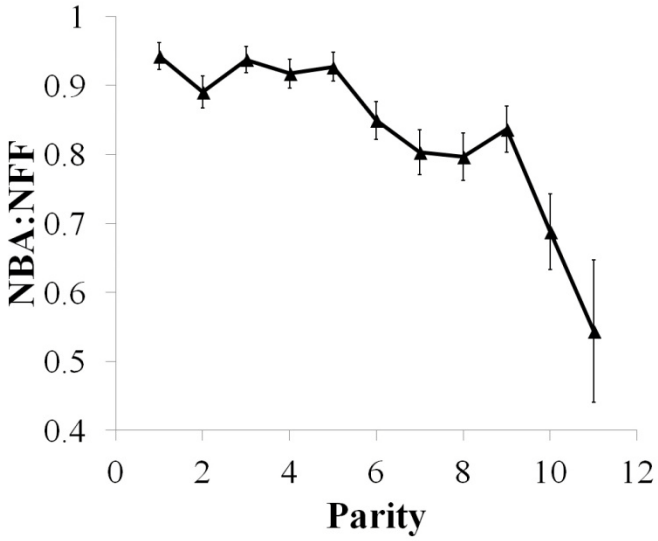

Figure 2.2. Fraction alive at birth estimated as number born alive (NBA) divided by number fully formed (NFF) across parities. Error bars denote SEM.

In the repeated measures analysis, there was a line × parity interaction for LWW ($P < 0.01$), due to greater litter weaning weights in MH mice versus ML mice at parity 6 ($P < 0.03$) and asymmetry of response at parity 7 due to greater litter weaning weights in MC mice compared to the average of the two selection lines ($P < 0.01$). Otherwise, line was not significant for any reproductive or maternal performance trait measured at a single parity.

Previous studies in these populations showed MH mice producing more pups than ML mice and found a positive genetic correlation between number born and heat loss (McDonald and Nielsen, 2007; Nielsen et al., 1997a). However, in those studies, litter size was only recorded for a single parity and pairs were older (12 wk) when mated compared to the current study (7 wk). These previous studies postponed breeding until mice reached full maturity while this study bred animals closer to onset of puberty to more closely imitate conditions in livestock systems. In previous studies, the difference in

litter size between MH and ML mice was small (1.6 pups) and there were small differences in litter size traits between selection lines at individual parities observed in this study, but these were insignificant when viewed in the context of lifecycle production. A previous study (McDonald and Nielsen, 2006) also found a tendency for MH mice to have heavier weaning weights compared to ML mice, which was not seen in the current study. In a study evaluating productivity and lifetime reproductive performance in mice, Newman et al. (1985a, b) found a decrease in litter size traits as parity increased, both at birth and weaning, analogous to the decrease in later parities in NFF, NW and FAB seen in this study. Johnston et al. (2007) also did not find a relationship between litter size and basal metabolic rate in mice. In pigs divergently selected for residual feed intake (RFI), Barea et al. (2010) found a positive association between RFI and heat production. In the same population of pigs, the high RFI line had smaller numbers of total piglets born, born alive, and weaned, indicating an inverse relationship between heat production and reproductive performance (Gilbert et al., 2007).

Pup weaning weight showed a tendency to increase in later parities ($P < 0.07$), due to the corresponding decrease in litter size (Fig. 2.3). Newman et al (1985b) evaluated lifetime productivity and reproductive performance in several lines of mice, and found a similar increase in PWW in later parities as the current study.

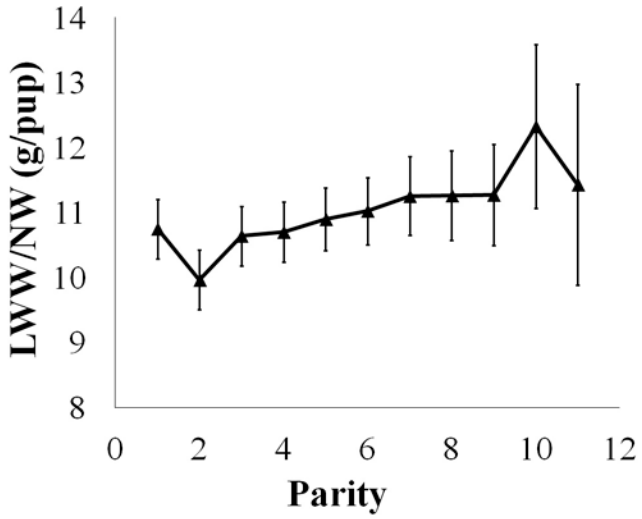

Figure 2.3. Average weaning weight per pup estimated as litter weaning weight (LWW) divided by number weaned (NW) across parities. Error bars denote SEM.

For lifetime production, there was evidence of asymmetry of selection for NW, LWW, and PWW ($P < 0.04$). MC mice weaned more pups over the span of their lifetime, as well as producing larger total weaning weights and larger weaning weights per pup than the average for the 2 selection lines (Fig. 2.4). Line was not significant for any other lifetime reproductive traits, and MH and ML mice did not differ in any trait.

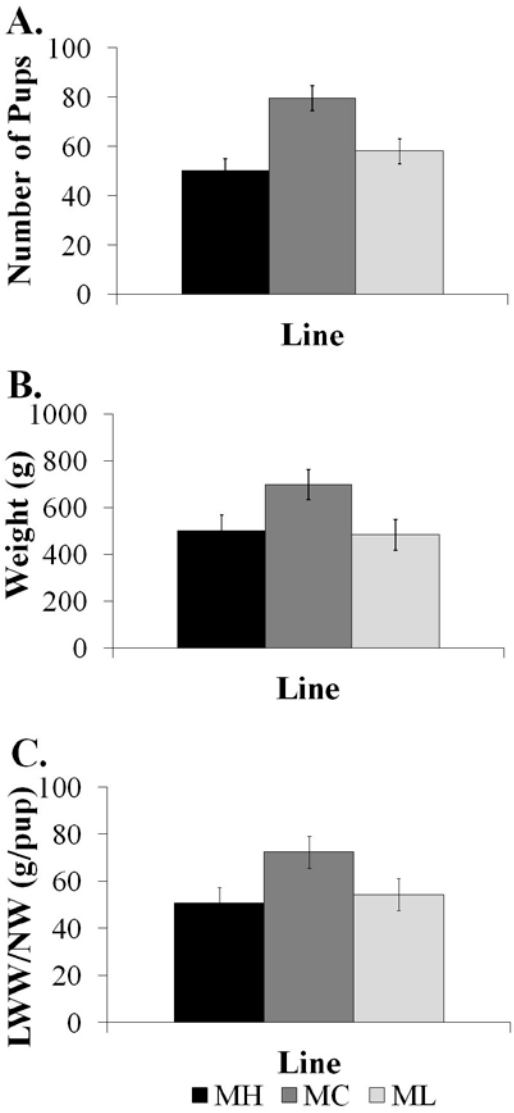

Figure 2.4. Lifetime number weaned (panel A), lifetime total weaning weight (panel B) and lifetime litter weaning weight/number weaned (panel C) per breeding pair for lines of mice selected for high (MH) or low heat loss (ML) or unselected control (MC). Error bars denote SEM.

Results of the linear regression analysis of birth interval are shown in Figure 2.5. Slopes for regression of birth interval on parity were 0.58 ± 0.14, 0.38 ± 0.12, and 0.06 ± 0.15 d/parity for MH, MC, and ML mice, respectively. Slopes were different between MH and ML mice (P = 0.01) with birth intervals increasing for MH mice in later parities while remaining similar for ML mice. Intercepts were 23.16 ± 1.04, 25.23 ± 1.04, and

24.54 ± 0.99 d for MH, MC, and ML mice, respectively. Intercepts were not significantly different between MH and ML mice (P = 0.18). Other studies in mice have also found that birth interval increased over parities (Wallinga and Bakker, 1978; Newman et al. 1985a). In both of these studies, birth interval was measured in multiple, independent lines and there were significant differences in birth interval between the various lines indicating that genetic differences are at least partially responsible. In the study by Wallinga and Bakker (1978), the increase in birth interval was more pronounced in mice that had been selected for increased litter size that were subjected to continuous mating instead of interval mating where the male was removed just before parturition and returned after weaning. Authors proposed that this was due to the stress associated with simultaneous gestation and lactation, which is amplified with larger litters. Dams could not meet the energetic needs of gestation while lactating so pregnancy was delayed, resulting in a longer breeding interval. Somewhat similarly, MH mice have a greater energetic requirement for maintenance that impedes pregnancy during lactation because the dams could not maintain concurrent gestation and lactation. A study by Gilbert et al. (2012) provides evidence to support this theory in pigs. Dams selected for low residual feed intake (that have also been shown to have lower maintenance energy requirements) were more able to divert energy to lactation by mobilizing body reserves than dams selected for high residual intake.

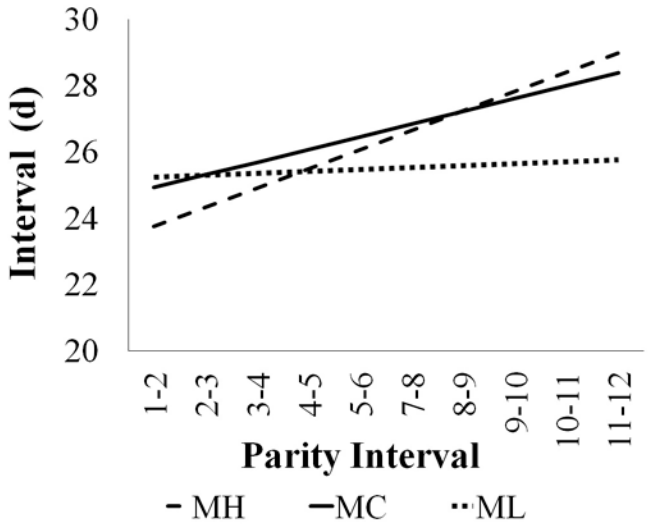

Figure 2.5. Linear relationship between birth interval and parity for lines of mice selected for high (MH) or low (ML) heat loss and the unselected control (MC).

Ultimately, selection for reduced maintenance energy did not result in substantial decreases in single-parity or lifetime reproductive performance, as there were few significant differences between MH and ML animals. When considering lifetime production, however, MC mice are superior to either selection line, particularly in weaning traits. This is in part due to the increased longevity of the MC line found in the survival analysis. Additionally, for the unselected control line, MC mice had lower rates of inbreeding, and thus accumulated inbreeding (F), than either selection line which were equal in accumulated inbreeding (F = 0.38 vs. 0.46). Inbreeding has repeatedly been shown to negatively affect traits associated with fitness, including reproductive and survival traits in several species, including mice (Falconer and MacKay, 1996). Studies have reported reduction in litter size, litter weight, and survival traits associated with an increase in inbreeding (Bowman and Falconer, 1960; Beilharz, 1982; DeRose and Roff, 1999). Therefore, less accumulated inbreeding is likely at least partially responsible for

the superior performance observed in MC mice. Because of the magnitude of the difference in feed intake previously observed in these populations, correlated response in reproductive performance is unlikely to outweigh benefits of reduced feed intake.

Survival analysis

Survival probabilities for all lines are shown in Figure 2.6. Control mice pairs had greatest survival rates at all time points, and the MH and ML lines showed different trends. The MH line appeared to have poorer survival rates during early parities, while ML mice survived well in early parities and were lost at a greater rate in later parities. This relationship was the justification for analyzing the data in 2 periods, as well as over the entire length of the study.

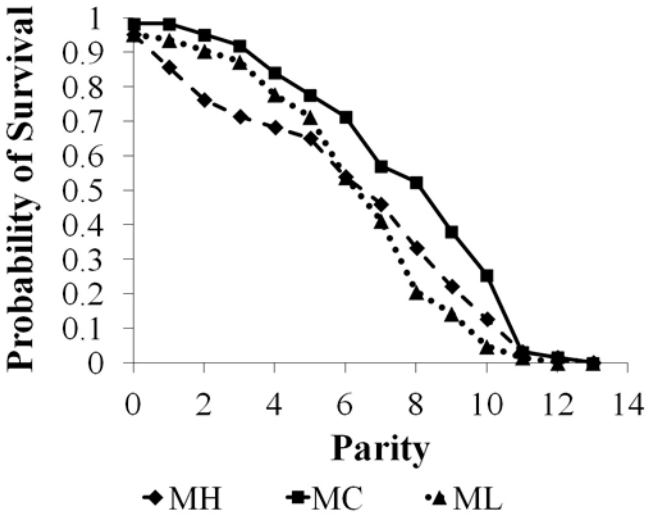

Figure 2.6. Probability of survival until next parity for breeding pairs of mice from lines selected for high (MH) or low (ML) heat loss and the unselected control (MC).

Hazard ratios, shown in Table 2.1, better quantify the relative risk of culling of one line to another. Over the entire study, MC breeding pairs had the smallest hazard and

were less likely to be culled than pairs of either selection line ($P < 0.03$). There was no difference in hazard rates between MH and ML lines ($P > 0.33$). However, MH mice had the greatest risk of culling before achieving their 5th parity, higher than both ML and MC mice ($P < 0.04$ and $P < 0.01$, respectively). In pairs that produced greater than 5 parities, ML mice were more likely to be culled than either MH mice or MC mice ($P < 0.04$ and $P < 0.01$, respectively).

Table 2.1. Hazard ratios comparing lines[1] for overall lifetime survival or for survival ≤ 5 parities or >5 parities

	Period		
Ratio	Overall	(≤ 5 parities)	(> 5 parities)
MH:MC	1.62* ± 0.13	3.49* ± 0.14	1.42 ± 0.19
ML: MC	2.00* ± 0.11	1.36 ± 0.36	2.58* ± 0.11
MH:ML	0.82 ± 0.17	2.57* ± 1.14	0.55* ± 0.15

[1]MH = high and ML = low heat loss selection; MC = unselected control

*Indicates a hazard ratio significantly different from 1 ($P < 0.05$)

Results indicate that MH mice are more likely to be culled early and ML later, but MC mice have greater survival rates overall. This outcome could have different implications, depending on the goals of the breeding system in question. For systems where smaller maximum parities are desirable, such as a nucleus population where a short generation interval is a priority, ML mice have less involuntary losses and thus enhanced overall reproductive efficiency in addition to their lower feed energy for maintenance. But this advantage erodes in systems allowing larger numbers of parities, where ML mice have greater rates of losses in later parities. Therefore, because nucleus operations are responsible for defining breeding objectives, reducing maintenance energy requirements may need to be balanced with longevity in breeding goals to prevent excessive losses of animals in commercial herds while still exploiting the improved feed

efficiency in all segments of the system. Notably, the increased reproductive survivability of MC mice is responsible for the observed lifetime reproductive performance improvement, and the greater numbers of weaned pups makes the MC line superior over either selection line in reproductive performance. The improvement in feed efficiency seen in ML mice may still prove to be substantial enough to override the increase in output seen in MC mice.

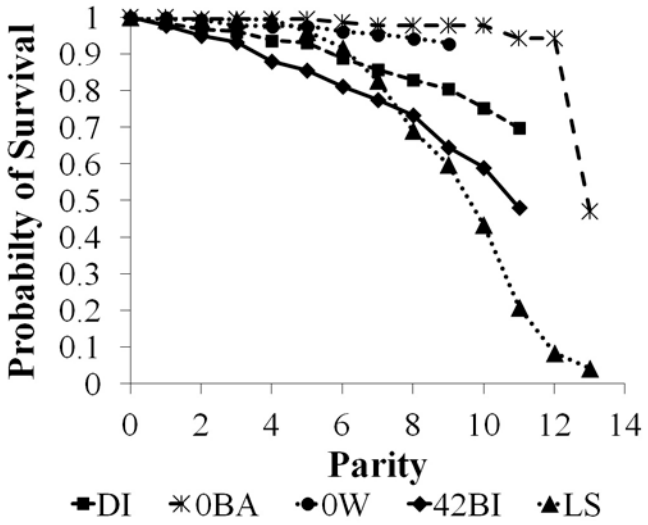

Figure 2.7. Probability of survival until next parity of breeding pairs of mice culled due to death or illness (DI), 2 consecutive letters with none born alive (0BA), 3 consecutive litters with none weaned (0W), a birth interval longer than 42 d (42BI), average of the most recent 2 litters was less than half the average of the first 3 (LS).

Table 2.2. Hazard ratios comparing lines[1] for overall lifetime survival within each culling criteria

	Culling Criteria[2]					
Ratio	DI	0BA	0W	42BI	LS	FL
MH:MC	2.66* ± 0.18	3.86 ± 0.30	2.04 ± 0.45	1.47 ± 0.23	1.10 ± 0.29	1.30 ± 0.98
ML:MC	2.36# ± 0.21	2.00 ± 0.73	2.36 ± 0.37	1.21 ± 0.29	1.89* ± 0.17	2.55 ± 0.47
MH:ML	1.13 ± 0.47	1.94 ± 2.34	0.86 ± 0.68	1.22 ± 0.44	0.58 ± 0.20	0.51 ± 0.49

[1]MH = high and ML = low heat loss selection; MC = unselected control
[2]DI = death or illness; 0BA = 2 consecutive litters with none born alive; 0W = 3 consecutive litters with none weaned; 42BI = birth interval longer than 42 d; LS = average of the most recent 2 litters was less than half the average of the first 3; FL = no first litter by 42 d cohabitation
*Indicates a hazard ratio significantly different from 1 ($P < 0.05$)
#Indicates a hazard ratio with a tendency to be different from 1 ($P < 0.10$)

Survival curves from the competitive risk analysis are shown in Figure 2.7. Survival rates were similar in the early stages of the study, but a long birth interval and small litter size became the most likely reason for culling in later parities. Table 2.2 shows the hazard ratios between lines for each culling criterion. The MH and ML mice did not differ for any criteria, but were more likely to be culled due to death or illness than MC mice ($P < 0.05$ and $P < 0.09$, for MH and ML vs. MC, respectively). Additionally, ML mice were more likely to be culled due to small litter size than MC mice ($P < 0.05$).

Parity equilibrium

Table 2.3 shows the mean number of parities for each line when the maximum number of allowed parities was 4, 6, 8, 10, or 12, as well as over the entire study period of 1 yr. When all animals were culled at 4, 6, and 8 parities, the average number of parities was greater for ML animals compared to MH animals. However, the average number of parities was greater for MH animals compared to ML animals when animals were maintained for a maximum of 10 and 12 parities and over a 1-yr time period. Control animals generally produced a greater number of parities than MH or ML animals at all time periods. Two exceptions occurred: MC average was lower than ML when a maximum of 6 parities was allowed, and MC average was lower than MH when a maximum of 10 parities was allowed.

Table 2.3. Average number of parities by line[1] assuming differing maximum number of parities allowed

Maximum	MH	MC	ML
4 parities	2.395	2.466	2.461
6 parities	3.290	3.368	3.383
8 parities	4.153	4.282	4.193
10 parities	4.945	4.933	4.841
12 parities	5.583	5.710	5.431
1 yr	6.214	6.248	6.036

[1]MH = high and ML = low heat loss selection; MC = unselected control

Similar to the results from survival analysis, the implications of these results depend on the strategy of the breeding program. The increased survival probabilities for ML mice in early parities resulted in a greater mean number of parities when animals are not retained for longer than 6 parities. Therefore, in breeding populations desiring a shorter generation interval, animals with low maintenance energy requirements not only offer the benefit of improved feed efficiency, but will also remain in the breeding population longer and potentially provide increased total output compared to high maintenance energy or unselected animals. In livestock breeding programs where longevity is important, such as a commercial population, the smaller mean number of parities seen in ML mice may be detrimental as it implies that in a population of animals selected for low maintenance energy requirements, replacement animals will have to brought in more frequently than in a population of high maintenance energy or unselected animals, to replace those that are involuntarily culled. This may increase the input costs because replacement animals must be obtained, and could erode the decreased input costs attributed to improved feed efficiency.

Implications

Selection for reduced heat loss and thus reduced maintenance energy requirements in mice has resulted in improved feed efficiency, which would be a desirable outcome in all livestock species. This study showed that reducing maintenance energy requirements did not negatively affect reproductive performance, as there were few significant differences in litter traits between high and low maintenance lines of animals. Low maintenance animals have better survival rates in early parities, which could increase their efficiency in systems where smaller maximum parities are desirable. However, their survival rates decline in later parities, and high maintenance energy animals showed improved longevity in systems allowing larger numbers of parities. Therefore, breeding objectives should be designed to balance reducing maintenance energy requirements while maintaining longevity. Additionally, animals not selected for reduced maintenance energy requirements showed improvements in lifetime weaning traits and overall survivability, partially due to less inbreeding in the MC line. Integration of these results with feed intake and efficiency data is necessary to determine the effect of changing maintenance energy requirements on lifetime efficiency.

Literature cited

Allison, P. D. 1997. Survival analysis using the SAS system. A practical guide. SAS Institute Inc., Cary, NC, USA.

Azzam, S. M., A. M. Azzam, M. K. Nielsen, and J. E. Kinder. 1990. Markov chains as a shortcut method to estimate age distributions in herds of beef cattle under different culling strategies. J. Anim. Sci. 68: 5-14.

Barea, R., S. Dubois, H. Gilbert, P. Sellier, J. van Milgen, and J. Noblet. 2010. Energy utilization in pigs selected for high and low residual feed intake. J. Anim. Sci. 88: 2062-2072.

Beilharz, R. G. 1982. The effect of inbreeding on reproduction in mice. Anim. Sci. 34: 49-54.

Bowman, J. C., and D. S. Falconer. 1960. Inbreeding depression and heterosis of litter size in mice. Gent. Res. 1: 262-274.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodal inference: A practical information-theoretic approach. Springer, New York.

DeRose, M. A., and D. A. Roff. 1999. A comparison of inbreeding depression in life-history and morphological traits in animals. Evolution 53: 1288-1292.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Pearson, Essex, England.

Ferrell, C. L., and T. G. Jenkins. 1985. Cow type and the nutritional environment: Nutritional aspects. J. Anim. Sci. 61: 725-741.

Gilbert, H., J.-P. Bidanel, Y. Billon, H. Lagant, P. Guillouet, P. Sellier, J. Noblet, and S. Hermesch. 2012. Correlated responses in sow appetite, residual feed intake, body

composition, and reproduction after divergent selection for residual feed intake in the growing pig. J. Anim. Sci. 90: 1097-1108.

Gilbert, H., J. P. Bidanel, J. Gruand, J. C. Caritez, Y. Billon, P. Guillouet, H. Lagant, J. Noblet, and P. Sellier. 2007. Genetic parameters for residual feed intake in growing pigs, with emphasis on genetic relationships with carcass and meat quality traits. J. Anim Sci. 85: 3182-3188.

Johnston, S. L., D. M. Souter, S. S. Erwin, B. J. Tolkamp, J. M. Yearsley, I. J. Gordon, A. W. Illius, I. Kyriazakis, and J. R. Speakman. 2007. Associations between basal metabolic rate and reproductive performance in C57BL/6J mice. J. Exp. Biol. 210: 65-74.

McDonald, J. M., and M. K. Nielsen. 2006. Correlated responses in maternal performance following divergent selection for heat loss in mice. J. Anim. Sci. 84: 300-304.

McDonald, J. M., and M. K. Nielsen. 2007. Renewed selection for heat loss in mice: Direct responses and correlated responses in feed intake, body weight, litter size, and conception rate. J. Anim. Sci. 85: 658-666.

Noblet, J., X. S. Shi, and S. Dubois. 1993. Metabolic utilization of dietary energy and nutrients for maintenance energy requirements in sows: Basis for a net energy system. Br. J. Nutr. 70: 407-419.

Newman, S., D. L. Harris, and D. P. Doolittle. 1985a. Lifetime parental productivity in twenty-seven crosses of mice. I. Birth traits. J. Anim. Sci. 61: 358-366.

Newman, S., D. L. Harris, and D. P. Doolittle. 1985b. Lifetime parental productivity in twenty-seven crosses of mice. II. Weaning traits reflecting reproduction and lactation. J. Anim. Sci. 61: 367-375.

Nielsen, M. K., B. A. Freking, L. D. Jones, S. M. Nelson, T. L. Vorderstrasse, and B. A. Hussey. 1997a. Divergent selection for heat loss in mice: II. Correlated responses in feed intake, body mass, body composition, and number born through fifteen generations. J. Anim. Sci. 75: 1469-1476.

Nielsen, M. K., L. D. Jones, B. A. Freking, and J. A. DeShazer. 1997b. Divergent selection for heat loss in mice: I. Selection applied and direct response through fifteen generations. J. Anim. Sci. 75: 1461-1468.

Wallinga, J. H., and H. Bakker. 1978. Effect of long-term selection for litter size in mice on lifetime reproduction rate. J. Anim. Sci. 46: 1563-1571.

Williams, C. B., and T. G. Jenkins. 2003. A dynamic model of metabolizable energy utilization in growing and mature cattle. I. Metabolizable energy utilization for maintenance and support metabolism. J. Anim. Sci. 81: 1371-1381.

Chapter III

Abstract

Changes in maintenance energy requirements and in feed efficiency have been achieved by divergent selection for heat loss in mice in 3 replicates, creating high (MH) and low (ML) lines, and an unselected control (MC). However, feed intake has mainly been measured in mature animals and not during growth or reproduction. Additionally, there is evidence that reducing maintenance energy will increase fat content, an undesirable result. In order to evaluate if selection has altered body composition and lifecycle feed intake, mating pairs were continuously mated and maintained for up to 1 yr unless culled. Offspring pairs were sampled from each line at each parity and maintained from 21 to 49 d of age. Feed intake was recorded for mating pairs throughout the year and on offspring pairs. Body weight (BW) was recorded on all animals at culling, as well as percent fat (PF), total fat (TF), and total lean (TL), measured by dual x-ray densitometry. Average daily gain (ADG) was also recorded for offspring. Energy partitioning was achieved using two approaches: Approach I regressed energy intake of the pair on sum of daily metabolic weight and total gain to obtain maintenance (b_m) and growth (b_g) coefficients for each line, replicate, feeding period, and sex (offspring pairs only); Approach II calculated b_m for each pair assuming constant energy values for lean and fat gain. Energy coefficients and body composition traits were evaluated for effect of selection (MH vs. ML) and asymmetry of selection ([MH + ML]/2 vs. MC). Both MC mating and offspring pairs tended to have larger BW than the average of the selection lines ($P < 0.08$). Males of offspring pairs weighed more than females ($P < 0.01$), while females of mating pairs weighed more than males ($P < 0.01$). Line was insignificant ($P >$

0.15) for body composition traits. Using Approach I, MH mice had a greater b_m than ML mice for mating pairs (P = 0.03), but not offspring pairs (P = 0.50). For Approach II, MH had a greater b_m than ML mice for both mating (P = 0.01) and offspring pairs (P = 0.01). The effect of selection for heat loss on body composition was smaller than previously reported and unlikely to outweigh the benefit of reduced feed intake, which was shown to be maintained throughout an entire lifecycle that included reproducing animals. Additionally, the reduction in energy intake seems primarily due to reduced maintenance energy costs, validating the success of the selection procedure.

Introduction

Maintenance energy requirements represent the largest input cost for all livestock species as a majority of metabolizable energy intake must meet these requirements. Reducing maintenance energy requirements independent of growth or other outputs (reproduction, growth, etc.) could greatly reduce feed intake and improve feed efficiency. Heat loss can be used to select for reduced maintenance energy requirements as energy that is consumed and not stored is released as heat, and this trait has previously been shown to exhibit genetic variation. Due to economic and management advantages, mice provide a useful model for livestock species to evaluate maintenance energy.

Nielsen et al. (1997b) demonstrated that selection response in maintenance energy requirements can be achieved by initiating divergent selection for heat loss in mice measured via direct calorimetry to create a high maintenance line (MH) and a low maintenance line (ML), as well as an unselected control (MC). Selection over a total of 25 generations has resulted in a 55.7% divergence (McDonald and Nielsen, 2007). A

correlated reduction in feed intake has been reported in the ML line when compared to the MH line (a 34% difference), and selection lines do not differ in body weight (McDonald and Nielsen, 2007; Nielsen et al., 1997a). However, there is evidence that ML mice have a higher percentage of body fat than MH mice (a 5.6% difference), an undesirable effect in livestock species (Kgwatalala and Nielsen, 2004; Nielsen et al., 1997a). Previous research in these lines has focused on young, mature mice (9 to 14 wk of age) and has not followed performance throughout an entire lifecycle, including continuously reproductive animals.

The objective of this study was to use these mouse lines to imitate a livestock system and determine if changing maintenance energy requirements affects body composition of young and adult mice and if improved feed efficiency of ML mice is maintained throughout an entire lifecycle.

Materials and methods

Experimental animals

All animal procedures were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Animals used in this study were sampled from lines of mice divergently selected for heat loss (MH = high, ML = low, MC = control) and have been previously described by Nielsen et al. (1997b). Briefly, heat loss per unit of metabolic body weight ($kcal \cdot kg^{-0.75} \cdot d^{-1}$) was measured on individual males 9 to 11 wk of age by placing them in direct calorimeters for 15 h overnight. Selection occurred in 3 replicates, creating 9 independent lines. Initial selection lasted for 16 generations, then selection was relaxed for 26 generations, though independence of the

lines was maintained. Selection was then resumed for 9 generations, based on the same selection criteria and lines have been maintained independently since, though no active selection has occurred. In the present study, which is part of a larger study on lifecycle efficiency of these lines, 21 mating pairs were selected from each line × replicate combination from generation 70, resulting in 189 total pairs of mice. Pairs were cohabitated (continuously mated) in plastic cages at 7 wk of age and maintained together for the duration of the study, unless culled, as described later. Data were collected from mating pairs and selected offspring produced throughout the experiment, which lasted one year unless culled due to illness, death, or poor reproductive performance. Rooms housing animals were subjected to a 12 h:12 h light:dark cycle and ambient temperature was maintained at 23.5 ± 1.0°C.

Data collection – mating pairs

Within each line × replicate combination, pairs were randomly separated into 3 groups of 7 pairs each for weekly feed intake measurements from weaning of one litter to weaning of the next (feeding periods). Feed intake was measured on group-1 animals from cohabitation until weaning of their first litter (period 1), group-2 animals were measured from weaning of parity 1 to weaning of parity 2 (period 2), group-3 animals from weaning of parity 2 to weaning of parity 3 (period 3), and then data collection returned to group-1 animals (period 4), etc. Feed intake measurements continued in this rotational matter for the year of the study or until pairs were culled, so that feed intake was measured on one third of the animals at any given feeding period. If the litter died before weaning, feed intake measurements were terminated when mortality was

observed. Body weights of each individual in the mating pair were measured at the beginning and end of the feed intake measurement period.

Pairs had ad libitum access to pelleted feed (Teklad diet 2019: 19% crude protein, 9.0% crude fat, 2.6% crude fiber, and 3.3 kcal of ME/g; Harlan Teklad, Madison, WI) in hanging wire baskets with weighted lids to minimize feed wastage. Consumption was measured by weekly feed disappearance.

Pairs were culled due to death or illness of either member, no first parity by 42 d cohabitation, 2 consecutive litters with none born alive, 3 consecutive litters with none weaned, 42 d between parities, or if the average size of the most recent 2 litters is less than half the average of the first 3 litters. Reproductive performance data were collected and relevant to the current study, weaning weights of the litter were collected for all mating pairs at every parity. Upon culling, a final body weight (g, BW) was recorded for each individual of a mating pair, and individuals were euthanized by CO_2 asphyxiation.

Body composition was measured on each individual of the mating pair at culling, except those culled for death or illness, as fat and protein content of those carcasses would be biased. Body composition was measured by dual x-ray densitometry (PIXImus, LUNAR Corporation, 726 Heartland Trail, Madison, WI) which measures bone mineral density and percent fat (PF), which can be used to calculate total fat mass (g fat mass excluding lean and bone content, TF), and total lean (g lean mass excluding fat and bone content, TL). In a pilot study on a subset of 22 animals, the correlation between dual x-ray prediction of body composition and chemical ether extraction measurements for percent fat was 0.88. Other studies comparing results from dual x-ray densitometry and chemical methods of measuring body composition have reported higher correlations and

accuracy when measuring more animals with a larger range of body weights (Brommage, 2003).

Data collection – offspring

Offspring were sampled at weaning (21 d) for data collection based on the group assigned to their parents, and animals identified for data collection followed a rotation in a similar manner to feeding period measurements of mating pairs. Offspring born in parity 1 were sampled from group-1 mating pairs to measure feed intake and post-weaning growth; offspring born in parity 2 were sampled from group-2 mating pairs; offspring born in parity 3 were sampled from group-3 mating pairs; and offspring sampling returned to group-1 mating pairs for parity 4. This rotation continued until culling of the mating pair. Two offspring of the same sex were selected from each available mating pair (4 male pairs and 3 female pairs, from the 7 parent mating pairs) for full feed intake and growth data collection (full data, FD), and the remaining pups of the opposite sex were kept to measure growth measurements only (reduced data, RD). If a mating pair in the group scheduled for data collection had been culled, an even male to female ratio was maintained when sampling from the remaining mating pairs. FD pups were housed together as a pair, and the RD pups were housed together with a maximum of 5 pups per cage.

FD offspring pairs had ad libitum access to a powdered feed (Teklad diet 2018: 18.6% crude protein, 6.2% crude fat, 3.5% crude fiber, and 3.1 kcal of ME/g; Harlan Teklad, Madison, WI) in glass jars with wire inserts to minimize feed wastage. Feed intake was measured weekly by feed disappearance from 21 d of age to 49 d of age (28 d

of measurements). Offspring on reduced data collection had ad libitum access to a pelleted diet (Teklad diet 2020X: 19.1% crude protein, 6.5% crude fat, 2.7% crude fiber, and 3.1 kcal of ME/g; Harlan Teklad, Madison, WI). Different diets were utilized for multiple reasons. Offspring require diets with a lower metabolizable energy content than mating pairs as they are not pregnant or lactating. For the FD animals, a powdered diet was used to reduce measurement error and the 2018 diet was more suitable as a powdered diet. In a pilot study, there was no difference in feed intake, growth and percent fat between mice kept on a pelleted or powdered diet (P = 0.97, 0.58, and 0.15, respectively), therefore the difference in methods between mating pair and offspring should not bias measurements.

Body weight was measured on FD cages as a pair at the beginning of data collection, and divided by 2 to estimate individual 21-d weights. Individual BW were taken at 49 d and used to calculate total gain and individual average daily gain (ADG, [49 d body weight – 21 d body weight]/28 d). Offspring in RD cages were weighed on a cage basis at 21 d and 49 d and divided by the total number of pups in the cage to estimate individual weights. These weights were used to calculate total gain and ADG identically to the FD cages. Average daily gain was measured on an individual basis rather than a cage basis to implement more accurate partitioning of feed intake, as described later. Offspring from the FD cages were also analyzed for body composition by dual x-ray densitometry, and PF, TF (g) and TL (g) were recorded for each individual.

Body composition analysis

Body composition was analyzed using the GLIMMIX procedure of SAS 9.3. All variables for both mating and offspring pairs were analyzed using the same model:

$$y_{ijkl} = line_i + sex_j + rep_k + line*rep_{ij} + line*sex_{ik} + rep*sex_{jk} + line*rep*sex_{ijk} + e_{ijkl},$$

where y_{ijkl} is the body composition trait recorded for each individual (PF, TF, or TL), $line_i$ is the fixed effect of line (MH, ML, or MC), sex_j is the fixed effect of the sex (male or female), and rep_k is the random effect of replicate (1, 2, or 3). For all traits, orthogonal contrasts were used to test for selection response (MH vs. ML) or asymmetry of response [(MH + ML)/2 vs. MC].

Estimation of maintenance energy costs

Maintenance energy costs for mating pairs and offspring pairs were obtained using similar methods. Total feed intake for each pair (mating pair or pair of offspring under FD collection) was calculated by summing weekly feed intake for each period the pair was undergoing feed intake measurements. The sum was converted to metabolizable energy by multiplying total feed (g) by 3.3 kcal/g for mating pairs and 3.1 kcal/g for offspring pairs (ME value provided by feed manufacturer). Offspring noted for feed wastage and with a high metabolizable energy intake to body weight gain ratio (consuming more than 1500 kcal while losing more than 10 g) were removed from the dataset (8 MH, 6 MC and 1 ML removed). Other mouse studies have suggested that selection could result in increased feed wastage, however relatively few animals were removed in this study, therefore this possibility was not further examined in this

population (Hastings et al., 1997). The sum of daily metabolic body weight ($\Sigma BW^{0.75}$) was calculated for each individual by the following method:

$$\Sigma BW^{0.75} = \text{integral of daily metabolic BW (kg)} = [(1.75*ADG)^{-1}]$$

$$*[(BW0+28*ADG)^{1.75} - BW0^{1.75}]$$

where BW0 is the body weight of the individual at the beginning of feed intake data collection and ADG is the average daily gain over the feeding period. The $\Sigma BW^{0.75}$ values for each individual in a mating or offspring pair were summed to obtain the total sum of daily metabolic weights on a cage basis.

Partitioning of total metabolizable energy intake was then achieved by two approaches utilized by Eggert and Nielsen (2006). In Approach I, energy was partitioned into components for maintenance and growth. Coefficients for maintenance (b_m, $kcal \cdot kg^{-0.75} \cdot day^{-1}$) and growth ($b_g$, kcal/g) were estimated for each replicate, line, and period combination using the following regression model in the REG procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC):

ME intake (kcal) = b_m ($\Sigma BW^{0.75}$) + b_g [total gain (g)] + b_w [weaning weight (g)] + error, where total gain for each cage was calculated as the sum of the ADG of each individual in the mating or offspring pair multiplied by the number of days under feed intake data collection. The coefficient for weaning weight was only present in analysis of mating pairs and is the weight of the litter produced by the mating pair in that specific replicate, line, and period combination. Coefficients were produced for each line × replicate × period (mating pairs) or line × replicate × sex × parity (offspring) combination. Consecutive feeding periods were combined into 3 total periods (TP, TP A: feeding periods 1 through 3; TP B: feeding periods 4 through 6; and TP C: feeding periods > 7) to

insure sufficient numbers within each line by replicate by TP grouping to accurately estimate coefficients.

In Approach II, maintenance coefficients were calculated for each pair, assuming costs for lean and fat deposition are constant amongst lines. Values for energy costs for lean (b_l) and fat (b_f) deposition of 2.9 kcal/g and 12.8 kcal/g, respectively were obtained from the literature (Pullar and Webster, 1977). The value for lean was derived from the value for protein gain reported (11.4 kcal/g), assuming lean is 25% protein and 75% water. The maintenance coefficient, b_m, was then calculated for each individual as:

$$b_m = \frac{\text{energy intake} - (b_f(\text{fat (g)}) + b_l(\text{lean (g)})}{\Sigma BW^{0.75}};$$

Fat gain and lean gain were obtained from the dual x-ray densitometry measurements taken at culling and were the sum of individual gains for each individual in a mating or offspring pair. For mating pairs, culling occurred at variable times and body composition was only measured at culling. Therefore, percentage of lean and fat was considered constant and used to estimate carcass composition at earlier feeding periods by multiplying percent fat by the total gain in each feeding period and then subtracting the fat gain from total gain to obtain lean gain.

Coefficients were then treated as a dependent variable and analyzed using the GLIMMIX procedure to evaluate differences due to line or sex, in the case of offspring. For Approach I, the following model was used for mating pairs:

$$y_{ijkl} = line_i + TP_j + line*TP_{ij} + rep_k + line*rep_{ik} + rep*TP_{jk} + e_{ijkl},$$

where y_{ijkl} is the coefficient for maintenance (b_m) or gain (b_g), $line_i$ is the fixed effect of line (MH, ML, or MC), TP_j is the fixed effect of the measurement period (A, B, or C), and rep_k is the random effect of replicate (1, 2, or 3).

For coefficients estimated for offspring under Approach I, the following model was used:

$$y_{ijkl} = line_i + sex_j + rep_k + line*rep_{ij} + line*sex_{ik} + rep*sex_{jk} + e_{ijkl},$$

where y_{ijkl} is the coefficient for maintenance (b_m) or gain (b_g), $line_i$ is the fixed effect of line (MH, ML, or MC), sex_j is the fixed effect of the sex of the offspring pair (male or female), and rep_k is the random effect of replicate (1, 2, or 3).

Because of the incomplete nature of data collection (e.g. group-1 had a data point at feeding period 1, 4, 7, and 10, but intake was not recorded during other feeding periods), feeding period was redefined again for Approach II. Similar to the analysis for Approach I, feeding periods were again grouped into total periods. Feeding periods 1 through 3 were grouped and designated TP A, feeding periods 4 through 6 were designated TP B, feeding periods 7 through 9 were designated TB C, and feeding periods 10 through 12 were designated TP D. This conversion insured that each mating pair had a feed intake measurement during each period until that mating pair was culled and created a balanced data set. For Approach II, the following model was used to analyze coefficients for mating pairs:

$$y_{ijkl} = line_i + TP_j + \beta ww_{ijkl} + rep_k + line*TP_{ij} + line*rep_{ik} + TP*rep_{jk} + line*TP*rep_{ijk} + pair(line*rep)_{ikl} + e_{ijkl},$$

where y_{ijkl} is the maintenance coefficient (b_m), $line_i$ is the fixed effect of line (MH, ML, or MC), $period_j$ is the fixed effect of the TP (A,B, C, and D), ww_{ijkl} is the weaning weight of the litter born to pair i in period j, β is the slope associated with the weaning weight covariate, rep_k is the random effect of replicate (1, 2, or 3), and pair(line*rep) is the random effect of the mating pair nested with line and replicate. Period was treated as a

repeated measure with pair nested within rep by line as the subject. An autoregressive variance component structure was applied to pair(rep*line) and chosen based on Akaike information criterion corrected for finite sample size (Burnham and Anderson, 2002).

For offspring, the following model was used to analyze maintenance coefficients obtained from Approach II:

$$y_{ijkl} = line_i + sex_j + rep_k + line*rep_{ij} + line*sex_{ik} + rep*sex_{jk} + line*rep*sex_{ijk} + e_{ijkl},$$

where y_{ijkl} is the coefficient for maintenance (b_m) or gain (b_g), $line_i$ is the fixed effect of line (MH, ML, or MC), sex_j is the fixed effect of the sex of the offspring pair (male or female), and rep_k is the random effect of replicate (1, 2, or 3).

For all models, orthogonal contrasts were used to test for selection response (MH vs. ML) or asymmetry of response [(MH + ML)/2 vs. MC].

Results and discussion

Body composition

Body composition results are shown in Table 3.1 (mating pairs) and Table 3.2 (offspring pairs). There were no differences between lines in TL, TF, or PF for mating pairs (P = 0.54, 0.15, and 0.77, respectively). Sex was significant for TL (P = 0.02) where females had more total lean content, however sex did not have an effect on any other body composition trait. The increase in TL in females was most likely related to in larger BW of females. Numerically, MC had the greatest TF, TL, and PF of the lines, mostly due to the larger overall body weight of these animals.

Table 3.1. Body composition of mating pairs by line[1] and sex[2]

	Percent Fat (%)	Total Fat (g)	Total Lean (g)
Line			
MC	23.52 ± 2.05	9.68 ± 0.99	30.52 ± 0.73
MH	21.53 ± 2.06	8.49 ± 0.99	29.76 ± 0.73
ML	21.85 ± 2.06	8.15 ± 0.99	28.20 ± 0.73
Sex[3]			
F	21.60 ± 1.34	8.67 ± 0.67	30.69[a] ± 0.51
M	23.00 ± 1.33	8.88 ± 0.67	28.30[b] ± 0.51

[1]MH = high and ML = low heat loss selection; MC = unselected control
[2]F = female; M = male
[3]Differing superscripts indicate a significant difference between females and males (P < 0.05)

Table 3.2. Body composition and growth of offspring pairs by line[1] and sex[2]

	Percent Fat (%)	Total Fat (g)	Total Lean (g)	Average Daily Gain (g/d)
Line				
MC	12.54 ± 0.45	3.33 ± 0.13	23.24 ± 0.61	0.61 ± 0.72
MH	12.01 ± 0.46	3.02 ± 0.14	22.20 ± 0.62	0.59 ± 0.75
ML	12.62 ± 0.45	3.14 ± 0.14	21.67 ± 0.62	0.56 ± 0.72
Sex[3]				
F	12.92[a] ± 0.35	2.92[a] ± 0.11	19.55[a] ± 0.44	0.47[a] ± 0.45
M	11.86[b] ± 0.34	3.41[b] ± 0.11	25.19[b] ± 0.44	0.70[b] ± 0.45

[1]MH = high and ML = low heat loss selection; MC = unselected control
[2]F = female; M = male
[3]Differing superscripts indicate a significant difference between females and males (P < 0.05)

No differences were detected due to line for TF, TL and PF in offspring (P = 0.24, 0.24, and 0.58, respectively). Sex was significant for all three traits (P < 0.01 for TF, P < 0.01 for TL, and P = 0.03 for PF). Males had greater amounts of TF and TL, however females had a larger PF. Nielsen et al. (1997a) showed ML mice to have a greater percent fat than MH mice, though the differences were numerically small (16.9% vs. 16.0%)

while Moody et al. (1997) found ML mice to have a greater percent fat than MH mice (a difference of 6.77% fat between lines). Kgwatalala and Nielsen (2004) found a slightly larger difference in fat percentage, with ML at 16.32% and MH at 14.45%, averaged across three different environmental temperatures. In all of these studies, measurements were taken at similar ages (9 to 14 wk), while age of mating pairs at analysis would range (15 to 55 wk) in the current study. This does not account for the lack of difference in offspring pairs, which were all 49 d of age when measured. It seems that differences in percent fat are less extreme when considering an entire life cycle.

Other studies have observed higher fat content in mice selected for improved feed efficiency, which can be related to the current study. Hastings et al. (1997) achieved divergence for maintenance energy requirements by selecting mice for food intake corrected by phenotypic regression on mean body weight at maturity. Authors found the low intake line to be 2.4 to 7.2% fatter than the high intake line, a much larger difference than what was reported in the current study though similar to some previous work in these heat loss lines (Bünger et al., 1998; Hastings et al., 1997; Moody et al., 1997).

Residual feed intake has been proposed a selection criterion that more accurately isolates maintenance energy requirements than traditional measures of feed efficiency. Barea et al. (2010) demonstrated that pigs selected for low residual feed intake had reduced heat production and thus lower maintenance energy requirements. A subsequent study on these lines found a tendency for a positive genetic correlation between residual feed intake and backfat and negative correlations with dressing percent and lean meat content (%), indicating that pigs with higher maintenance energy requirements may have a higher fat content, which contradicts the hypothesis that lower maintenance animals

have a higher fat content, but reflects the lack of differences in body composition seen in this study (Gilbert et al., 2007). However, it should be noted that calculation of residual feed intake in the French pig study accounted for backfat and may prevent an increase in fat content with lower maintenance requirements than selection for reduced maintenance energy requirements alone. Richardson et al. (2001) also found evidence of increased fat content with increased residual feed intake after a single generation of selection in beef cattle.

Final body weight

Differences between lines in final body weight of mating pairs tended toward significance (P = 0.08), and there was significant evidence of asymmetry of selection (P = 0.04) because the MC mice weighed more than the average of the selection lines (Fig 1). Sex was highly significant (P < 0.01), with females weighing more than males at culling (44.2 ± 0.81 g vs. 40.6 ± 0.81 g). Kgwatalala and Nielsen (2004) observed higher body weights in males compared to females from this population, however mice were 9 wk of age or younger in that study and females were not reproducing. There was no significant line by sex interaction (P = 0.20).

Selection effect of final body weight of offspring was not significant (P = 0.17), however there was a tendency towards asymmetry (P = 0.07), with MC mice again being heavier (Fig 3.1). Sex was again highly significant (P < 0.01), however in this case males were heavier than females (31.3 ± 0.54 vs. 24.7 ± 0.54). Again, the line by sex interaction was insignificant (P = 0.48). This is in agreement with the results seen by Kgwatalala and Nielsen (2004). Average daily gain (Table 3.2) was not different due to line (P = 0.49),

however males gained more per day than females (P < 0.01). Similar results in growth across lines were also reported in other studies using these lines (Kgwatalala and Nielsen, 2004; Murphy et al., 2013).

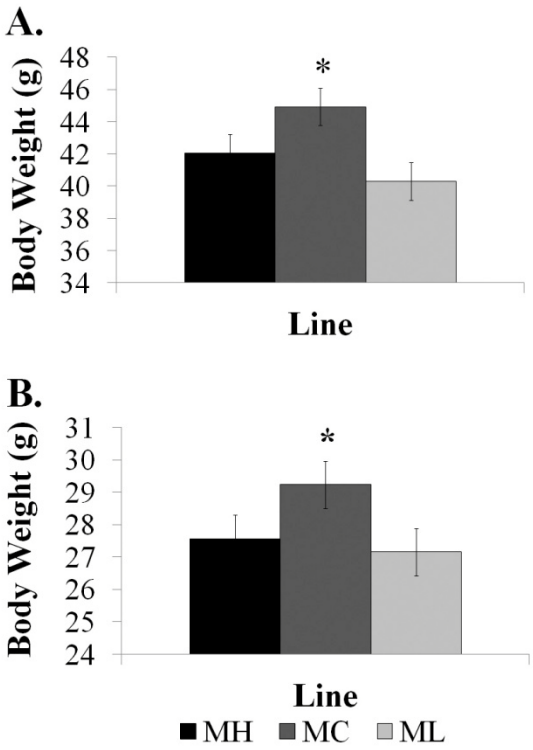

Figure 3.1. Final body weight (g) of mating pairs (A) and offspring pairs (B) for lines of mice selected for high (MH) or low heat loss (ML) or unselected control (MC). Significant asymmetry of selection, measured by orthogonal contrasts ([MH + ML]/2 – MC), indicated by * (P < 0.10).

In lines of mice selected for feed intake adjusted for body weight, Hastings et al. (1997) did not observe a strong relationship between selection and body weight, though the high line did tend to have 7 to 11% greater body weights averaged over 3 generations of selection. However, there were no significant differences in body weight at 70 or 98 d of age in 10-wk old males sample from the 29 or 30th generation of selection. Control animals were of intermediate body weight at 70 d of age and heavier than both the high and low line at 98 d of age, though the difference was not significant. Conversely, MC

mice have consistently been reported as heavier in previous studies of the populations described in the current study (Kgwatalala and Nielsen, 2004; McDonald and Nielsen, 2007; Murphy et al., 2013).

After a single generation of selection for residual feed intake in beef cattle, which one would expect to be similar to selection for maintenance energy requirements, there were no differences between animals from the high and low line (Richardson et al., 2001). However, the correlated response in body weight to selection for residual feed intake in pigs was inconsistent with results observed in the current study. In pigs, high residual feed intake animals also had larger body weights than low residual feed intake animals after 6 wk, despite having similar weaning weights (Barea et al., 2010).

The higher female body weights observed in mating but not offspring pairs is likely due to the fact that these females were reproductively active and produced multiple parities. Increased female growth with multiple parities has been observed in several mouse studies (Brien and Hill, 1986; Rebholz et al., 2012). In lines of mice with the lit/lit mutation, which is believed to reduce production of growth hormone, growth rates and mature body size are substantially decreased compared to normal mice. Female lit/lit mice subjected to repeated pregnancies were able to overcome this effect and achieved greater growth rates than heterozygous mice (Beamer and Eicher, 1976). Both lit/lit mice and heterozygous mice subjected to repeated pregnancies grew more than unmated controls, indicating that changes in growth hormone levels during pregnancy (among other hormonal changes) cause increased growth in females. Brien and Hill (1986) measured female growth across multiple parities in several lines selected for different criteria, and showed variation between these lines. Variation in growth rates of older,

unmated males and females in the MH and ML lines has not been studied. However, due to the nature of the lifecycle evaluation that was the ultimate goal of the present experiment, it could not be accurately analyzed in the current study.

Lifecycle feed intake

Energy coefficients for mating pairs are reported in Table 3. In Approach I for mating pairs, line tended to have an effect on b_m ($P = 0.06$), but was insignificant for b_g ($P = 0.86$). For b_m, there was evidence of a selection response ($P = 0.03$), but no evidence of asymmetry of selection ($P = 0.24$). Feeding period was insignificant for b_m and tended to be significant for b_g ($P = 0.11$ and $P = 0.06$, respectively). MH mating pairs consumed about 16% more for maintenance ($kcal \cdot kg^{-0.75} \cdot day^{-1}$) than ML pairs, and MC animals were intermediate. In Approach II, line had a significant effect on b_m ($P = 0.03$) due to a significant selection response ($P = 0.01$). Similar to Approach I, MH mice consumed 20% $kcal \cdot kg^{-0.75} \cdot day^{-1}$ more for maintenance than ML mice, and MC mice were intermediate. Surprisingly, the correlation between successive measurements on the same mating pair was low (0.12), and authors cannot account for this result. Overall, the reduced feed intake for maintenance seen in younger animals from these lines is maintained in mature, reproducing mice. Gilbert et al. (2012) also found that pigs selected for reduced residual feed intake produced dams with lower daily feed intake and residual feed intake as dams during gestation and lactation. Using values from Approach II for a 40 g male and 44 g female, MC pairs would consume 308.9 g, MH would consume 358.24 g, and ML 298.6 g of feed for maintenance over a 28-d period.

Table 3.3. Energy coefficients by line[1] for mating pairs using 2 approaches[2]

	Approach I		Approach II
Line	b_m[3*]	b_g[3]	b_m[3*]
MC	161.39 ± 11.49	2.40 ± 1.96	233.34 ± 14.56
MH	183.14 ± 11.49	0.97 ± 1.96	270.65 ± 14.90
ML	157.83 ± 11.49	1.19 ± 1.96	225.62 ± 16.41

[1]MH = high and ML = low heat loss selection; MC = unselected control

[2]Approach I simultaneously estimated maintenance and gain costs; approach II assumed common costs for lean and fat gain and estimated maintenance costs

[3]b_m = maintenance coefficient (kcal·kg$^{-0.75}$·day^{-1}); b_g = total gain coefficient (kcal/g)

[*]Indicates significant response to selection ($P < 0.05$), evaluated using orthogonal contrasts (MH – ML)

Energy coefficients for offspring pairs are reported in Table 3.4. For offspring, both b_m and b_g were not different across lines ($P = 0.50$ and $P = 0.98$, respectively) using Approach I though MH mice did consume 7% more feed than ML mice. Sex was also insignificant for both traits ($P = 0.80$ for b_m and $P = 0.45$ for b_g). However, under Approach II, line was significant ($P = 0.01$) and females tended to have a greater b_m than males (a 6% difference, $P = 0.06$). There was a significant response to selection ($P < 0.01$) and a tendency for asymmetry of response ($P = 0.06$). MH mice consumed 16% more for maintenance (kcal·kg$^{-0.75}$·day^{-1}) than ML mice, and MC mice were intermediate, but more similar to ML mice. Using Approach II values and starting with 11.0-g males, two MC males would consume 268.0 g, two MH males would consume 295.8 g, and two ML males would consume 261.8 g of feed over a 28-d period. Starting with 10.9-g females, two MC females would consume 246.8 g, two MH females would consume 272.8 g, and two ML females would consume 236.8 g of feed over a 28-d period.

Table 3.4. Energy coefficients by line[1] and sex[2] for offspring pairs using 2 approaches[3]

	Approach I		Approach II
	b_m[4]	b_g[4]	b_m[4*#]
Line			
MC	254.19 ± 22.48	1.31 ± 2.30	228.99 ± 7.47
MH	271.03 ± 22.48	1.49 ± 2.30	261.88 ± 7.60
ML	237.13 ± 22.48	1.82 ± 2.30	225.81 ± 7.59
Sex			
F	249.95 ± 21.91	4.27 ± 1.41	246.03 ± 6.85
M	258.29 ± 21.91	1.55 ± 1.41	231.75 ± 6.80

[1] MH = high and ML = low heat loss selection; MC = unselected control
[2]F = female; M = male
[3]Approach I simultaneously estimated maintenance and gain costs; approach II assumed common costs for lean and fat gain and estimated maintenance costs
[4]b_m = maintenance coefficient ($kcal \cdot kg^{-0.75} \cdot day^{-1}$); b_g = total gain coefficient (kcal/g)
[*]Indicates significant response to selection ($P < 0.05$), evaluated using orthogonal contrasts (MH – ML)
[#]Indicates significant difference due to sex ($P < 0.05$)

Previous analysis of feed intake in these lines has only been done on young, non-reproducing animals, therefore comparison with offspring pair data is most appropriate. Eggert and Nielsen (2006) used similar approaches to estimate coefficients for maintenance and growth as employed here. Maintenance coefficients estimated here were greater than those estimated by Eggert and Nielsen using Approach I while growth coefficients were smaller. Due to the nature of regression analysis, this is not an unusual result as errors in estimated coefficients would be expected to be negatively correlated. If one coefficient is larger, the other must necessarily be smaller. The difference between MH and ML mice in maintenance energy costs was similar between the current study and

the Eggert and Nielsen (2006) study (7% vs. 9%, respectively). Maintenance coefficients estimated in Approach II were also greater than those estimated in a similar method by Eggert and Nielsen. However, in both studies MH mice required about 16% more energy for maintenance than ML mice. Eggert and Nielsen also found females to require 7% more energy for maintenance than males.

In other studies with this population, feed intake differences between MH and ML lines were larger (20 to 34%) (McDonald and Nielsen, 2007; Murphy et al., 2013; Nielsen et al., 1997a). However, feed intake was analyzed as grams of feed intake per unit body weight (or body weight raised to the 0.75 power) rather than kilocalories of metabolizable energy and not as coefficients calculated by regression as in this study. Additionally, in previous studies animals on feed intake were housed individually rather than as pairs. Individual animals would presumably require more energy to maintain body temperature than those housed as a pair. Furthermore, due to the fact that this study is part of a larger, lifetime efficiency study, feed intake measurements were done on a cage basis. In the case of mating pairs, cages included mature animals of both sexes as well as the litter produced, which would begin consuming feed as they approached weaning age. Measurements on a cage basis clearly do not facilitate partitioning feed intake amongst all the animals in the cage, but do provide input values for a lifetime efficiency evaluation that is mostly focused on total consumption of all animals in the system at any given life stage.

Other studies in mice have shown that lower heat production is associated with reduced feed intake or improved feed efficiency (Archer and Pitchford, 1996; Hastings et al., 1997; Selman et al., 2001). Hughes and Pitchford (2004) measured feed intake in

mice selected for post-weaning net feed intake (a similar trait to residual feed intake) throughout pregnancy and lactation to determine the effect of these processes on feed intake divergence. Divergence was maintained during pregnancy but lines converged for 2-wk period in early lactation, immediately following parturition. This convergence could be occurring for MH and ML mice and could partially explain the smaller difference in feed intake observed in the current study compared to previous studies utilizing these lines. Low heat production is also associated with reduced residual feed intake in chickens and beef cattle (Gabarrou et al., 1998; Luiting et al., 1991; Nkrumah et al., 2006). Barea et al. (2010) selected for high and low residual feed intake in swine and observed reduced total and fasting heat production in the low line.

The underlying cause of the differences in maintenance energy requirements between MH and ML lines has been previously studied. Differences in activity levels were determined to account for 11.5% of the differences in heat loss and 17 to 36% of the difference in feed intake between lines, with MH mice being more active (Mousel et al., 2001; Sojka et al., 2013). Differences in mitochondrial efficiency have also been found to be partially responsible for divergence in heat production, mainly due to increased uncoupling during electron transport in MH mice (McDonald et al., 2009; Murphy et al., 2013).

Implications

Selection for reduced heat loss to reduce maintenance energy requirements has been previously shown to improve feed efficiency in mice. In this study, the reduced energy intake was confirmed in post-weaning offspring and also shown to be maintained

in older, mating individuals. Therefore, the reduced maintenance energy requirement and resulting improved feed efficiency is maintained throughout all life stages. However, this study did not confirm higher fat content of low maintenance animals seen in previous studies of these populations. Therefore, reducing maintenance energy requirements may not negatively affect body composition and selection should result in animals with improved feed efficiency throughout their entire life cycle. Integration of these results with reproductive performance and longevity is necessary to determine the overall effect of changing maintenance energy requirements on lifetime efficiency.

Literature Cited

Archer, J. A., and W. S. Pitchford. 1996. Phenotypic variation in residual food intake of mice at different ages and its relationship with efficiency of growth, maintenance and body composition. Anim. Sci. 63: 149-157.

Barea, R., S. Dubois, H. Gilbert, P. Sellier, J. van Milgen, and J. Noblet. 2010. Energy utilization in pigs selected for high and low residual feed intake. J. Anim. Sci. 88: 2062-2072.

Beamer, W. G., and E. M. Eicher. 1976. Stimulation of growth in the little mouse. J. Endocrinol. 71: 37-45.

Brien, F. D., and W. G. Hill. 1986. Reproductive performance over repeated parities of lines of mice selected for appetite, lean growth and fatness. Anim. Sci. 42: 397-410.

Brommage, R. 2003. Validation and calibration of DEXA body composition in mice. Am. J. Physiol. Endocrinol. Metab. 285: E454-E459.

Bünger L., M. G. MacLeod, C. A. Wallace, and W. G. Hill. 1998. Direct and correlated effects of selection for food intake corrected for body weight in the adult mouse. Proc. 6th World Congr. Genet. Appl. Livest. Prod., Armidale 26: 97-100.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodal inference: A practical information-theoretic approach. Springer, New York.

Eggert, D. L., and M. K. Nielsen. 2006. Comparison of feed energy costs of maintenance, lean deposition, and fat deposition in three lines of mice selected for heat loss. J. Anim. Sci. 84: 276-282.

Gabarrou, J. F., P. A. Geraert, N. Francois, S. Guillaumin, M. Picard, and A. Bordas. 1998. Energy balance of laying hens selected on residual food consumption. Br. Poult. Sci. 39: 79-89.

Gilbert, H., J.-P. Bidanel, Y. Billon, H. Lagant, P. Guillouet, P. Sellier, J. Noblet, and S. Hermesch. 2012. Correlated responses in sow appetite, residual feed intake, body composition, and reproduction after divergent selection for residual feed intake in the growing pig. J. Anim. Sci. 90: 1097-1108.

Gilbert, H., J. P. Bidanel, J. Gruand, J. C. Caritez, Y. Billon, P. Guillouet, H. Lagant, J. Noblet, and P. Sellier. 2007. Genetic parameters for residual feed intake in growing pigs, with emphasis on genetic relationships with carcass and meat quality traits. J. Anim. Sci. 85: 3182-3188.

Hastings, I. M., S. M. Moruppa, L. Bünger, and W. G. Hill. 1997. Effects of selection on food intake in the adult mouse. J. Anim. Breed. Genet. 114: 419-434.

Hughes, T. E., and W. S. Pitchford. 2004. How does pregnancy and lactation affect efficiency of female mice divergently selected for post-weaning net feed intake? Aust. J. Exp. Agr. 44: 501-506.

Kgwatalala, P. M., and M. K. Nielsen. 2004. Performance of mouse lines divergently selected for heat loss when exposed to different environmental temperatures. II. Feed intake, growth, fatness, and body organs. J. Anim. Sci. 82: 2884-2891.

Luiting, P., J. W. Schrama, W. van der Hel, and E. M. Urff. 1991. Metabolic differences between white leghorns selected for high and low residual food consumption. Br. Poult. Sci. 32: 763-782.

McDonald, J. M., and M. K. Nielsen. 2007. Renewed selection for heat loss in mice: Direct responses and correlated responses in feed intake, body weight, litter size, and conception rate. J. Anim. Sci. 85: 658-666.

Moody, D. E., D. Pomp, and M. K. Nielsen. 1997. Variability in metabolic rate, feed intake and fatness among selection and inbred lines of mice. Genet. Res. 70: 225-235.

Mousel, M. R., W. W. Stroup, and M. K. Nielsen. 2001. Locomotor activity, core body temperature, and circadian rhythms in mice selected for high or low heat loss. J. Anim. Sci. 79: 861-868.

Murphy, T. W., J. M. McDonald, and M. K. Nielsen. 2013. Hepatic mitochondrial efficiency in lines of mice differing in feed intake. J. Anim. Sci. 91: 2077-2082.

Nielsen, M. K., B. A. Freking, L. D. Jones, S. M. Nelson, T. L. Vorderstrasse, and B. A. Hussey. 1997a. Divergent selection for heat loss in mice: II. Correlated responses in feed intake, body mass, body composition, and number born through fifteen generations. J. Anim. Sci. 75: 1469-1476.

Nielsen, M. K., L. D. Jones, B. A. Freking, and J. A. DeShazer. 1997b. Divergent selection for heat loss in mice: I. Selection applied and direct response through fifteen generations. J. Anim. Sci. 75: 1461-1468.

Nkrumah, J. D., E. K. Okine, G. W. Mathison, K. Schmid, C. Li, J. A. Basarab, M. A. Price, Z. Wang, and S. S. Moore. 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methande production, and energy partitioning in beef cattle. J. Anim. Sci. 84: 145-153.

Pullar, J. D., and A. J. F. Webster. 1977. The energy cost of fat and protein deposition in the rat. Brit. J. Nutr. 37: 355-363.

Richardson, E. C., R. M. Herd, V. H. Oddy, J. M. Thompson, J. A. Archer, and P. F. Arthur. 2001. Body composition and implication for heat production of Angus steer progeny of parents selected for and against residual feed intake. Aust. J. Exp. Agr. 41: 1065-1072.

Selman, C., S. Lumsden, L. Bunger, W. G. Hill, and J. R. Speakman. 2001. Resting metabolic rate and morphology in mice (mus musculus) selected for high and low food intake. J. Exp. Biol. 204: 777-784.

Sojka, P. A., R. S. Griess, and M. K. Nielsen. 2013. Locomotor activity and body temperature in selected mouse lines differing greatly in feed intake. J. Anim. Sci. 91: 3557-3563.

Chapter IV

Abstract

Divergent selection for heat loss was conducted in 3 independent replicates of a high (MH) and low (ML) line, and unselected control (MC). Improvement in feed efficiency was observed in ML mice due to a reduced maintenance energy requirement, but there was also a slight decline in reproductive performance, survivability, and body composition, particularly when compared to MC animals. Therefore, the objective of this study was to model a lifecycle scenario similar to a livestock production system and calculate total inputs and outputs to estimate overall biological efficiency of these lines and determine if reduced feed intake resulted in improved lifecycle efficiency. Feed intake, reproductive performance, growth, and body composition were recorded on 21 mating pairs from each line by replicate combination, cohabitated at 7 wk of age and maintained for up to 1 yr unless culled. Proportion of animals at each parity was calculated from survival rates estimated from previous research when enforcing a maximum of 4, 8, or 12 allowed parities. This parity distribution was then combined with values from previous studies to calculate inputs and outputs of mating pairs and offspring produced in a single cycle at equilibrium. Offspring output was defined as kg of lean output of offspring at 49 d. Offspring input was defined as Mcal of energy intake for growing offspring from 21 to 49 d. Parent output was defined as kg of lean output of culled parents. Parent input was defined as Mcal of energy intake for mating pairs from weaning of one parity to weaning of the next. Offspring output was greatest in MC mice, due to superior body weight and numbers weaned, while output was lowest in ML mice due to smaller litter sizes and lean content. Parent output did not differ substantially

between lines, but was greatest in MH mice due to poorer survival rates resulting in more culled animals. Input was greatest in MH and lowest for ML mice for both offspring and parent pairs, consistent with previous results in these lines. Lifecycle efficiency was similar in MC and ML mice, while MH mice were least efficient. Ultimately, superior output in MC mice slightly outweighed the decrease in inputs resulting from decreased maintenance energy requirements in ML animals. Therefore, selection to reduce maintenance energy requirements may be more useful in terminal crosses or in a selection index in order to reduce possible negative effects on output, especially reproductive performance.

Introduction

Reducing maintenance energy requirements in livestock species is an appealing goal, as a majority of feed intake is consumed to meet these requirements. Furthermore, a reduction in maintenance energy requirements should reduce feed intake without affecting growth or production, thus improving feed efficiency (Williams and Jenkins, 2003). Heat loss can be used as an indicator trait for maintenance energy requirements as energy that is consumed and not stored is released as heat.

Nielsen et al. (1997b) successfully initiated selection for heat loss measured via direct calorimetry in mice, creating high (MH) and low (ML) maintenance lines, along with an unselected control (MC). As expected, ML mice consistently consume less feed per unit body weight than MH mice (McDonald and Nielsen, 2007; Nielsen et al., 1997a). However, several correlated responses to selection have been noted in this population that could diminish the improvement in feed efficiency, especially across an entire lifecycle.

Decreased lean content, litter size, and weaning weight have been observed in ML mice (McDonald and Nielsen, 2007; Nielsen et al., 1997a). Yet, when these mice were examined across several life-stages, including reproductive animals, these differences were less extreme and MC mice actually have superior productivity and reproductive performance (Bhatnagar and Nielsen, 2014a,b). Control mice also have greater overall survival rates, though rates were greatest in ML mice when only allowed to produce up to 5 parities (Bhatnagar and Nielsen, 2014b). Thus, benefits of selecting for reduced maintenance energy requirements could differ depending on how long mating animals are maintained.

The objective of this study was to use these mouse lines to imitate a livestock system and determine if differences in reproductive performance, productivity, survivability, and body composition outweigh improved individual feed efficiency observed in animals with lower maintenance energy requirements.

Materials and methods

Experimental animals

All animal procedures were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Animals used in this study were sampled from lines of mice divergently selected for heat loss as an indicator of maintenance energy requirements (MH = high, ML = low, MC = control) and have been previously described by Nielsen et al. (1997b). Briefly, heat loss per unit of metabolic body weight ($kcal \cdot kg^{-0.75} \cdot day^{-1}$) was measured on individual males 9 to 11 wk of age by placing them in direct calorimeters for 15 h overnight. Selection occurred in 3 replicates, creating 9

independent lines. Initial selection lasted for 16 generations, then selection was relaxed for 26 generations, though independence of the lines was maintained. Selection was then resumed for 9 more generations, based on the same selection criteria. In the present study, 21 mating pairs were selected from each of the 9 line by replicate combinations from generation 70, resulting in 189 total pairs of mice. Pairs were cohabitated at 7 wk of age and maintained together for the duration of the study, unless culled. Within each line by replicate combination, pairs were randomly separated into 3 groups of 7 pairs. Mating pairs were housed in plastic cages with wire lids and had ad libitum access to water and feed (Teklad diet 2019: 19% crude protein, 9.0% crude fat, 2.6% crude fiber, and 3.3 kcal of ME/g; Harlan Teklad, Madison, WI). Offspring were sampled at weaning (21 d) and maintained until maturity (49 d) for data collection based on the group assigned to their parents (offspring born in parity 1 were sampled from group-1 mating pairs; offspring born in parity 2 were sampled from group-2 mating pairs; offspring born in parity 3 were sampled from group-3 mating pairs; and offspring sampling returned to group-1 mating pairs for parity 4). Offspring pairs had ad libitum access to a either a powdered feed (Teklad diet 2018: 18.6% crude protein, 6.2% crude fat, 3.5% crude fiber, and 3.1 kcal of ME/g; Harlan Teklad, Madison, WI) or a pelleted diet (Teklad diet 2020X: 19.1% crude protein, 6.5% crude fat, 2.7% crude fiber, and 3.1 kcal of ME/g; Harlan Teklad, Madison, WI). Feed intake for offspring was only recorded for those fed the powdered diet, and in a pilot study, there was no difference in feed intake between pelleted and powdered diet ($P = 0.97$). Rooms housing animals were subjected to a 12 h:12 h light:dark cycle and ambient temperature was maintained at 23.5 ± 1.0°C. Culled animals were euthanized by CO_2 asphyxiation.

Performance data

Complete description of collection and analysis of performance data has been reported by Bhatnagar and Nielsen (2014a,b), but will be briefly described here. Feed intake was measured weekly on a rotational basis depending on group. In mating pairs, feed intake was measured on group-1 animals from cohabitation until weaning of their first litter (period 1), group-2 animals were measured from weaning of parity 1 to weaning of parity 2 (period 2), group-3 animals from weaning of parity 2 to weaning of parity 3 (period 3), and then feed intake collection returned to group-1 animals (period 4), etc. For feed intake measurement in offspring pairs, mice born in parity 1 were sampled from group-1 mating pairs; offspring born in parity 2 were sampled from group-2 mating pairs; offspring born in parity 3 were sampled from group-3 mating pairs; and offspring sampling returned to group-1 mating pairs for parity 4 and feed intake was measured from 21 to 49 d of age. Linear regression was used to estimate coefficients for maintenance (b_m, $kcal \cdot kg^{-0.75} \cdot d^{-1}$) and weaning weight ($b_w$, kcal/g). Coefficients for lean and fat growth (b_l =2.9 and b_f =12.8, respectively) were obtained from the literature (Pullar and Webster, 1977). Values obtained for b_m are shown in Table 4.1.

Table 4.1: Maintenance energy coefficients (b_m, kcal·BW$^{-0.75}$·day^{-1}) for offspring and parents by line[1], sex[2], and feeding period[3]

	Line		
	MH	MC	ML
Offspring Maintenance Coefficient			
Sex			
M	253.47	222.44	219.35
F	270.30	235.54	232.26
Parent Maintenance Coefficient			
Feeding Period			
1	266.25	237.95	247.11
2	276.70	241.01	236.32
3	249.42	238.85	218.78
4	290.23	215.56	200.28

[1] MH = high and ML = low heat loss selection; MC = unselected control
[2]M = male; F = female
[3]Feeding period 1 = parity 1 to 3; feeding period 2 = parity 4 to 6; feeding period 3 = parity 7 to 9; feeding period 4 = parity 10 to 12

Body weight (BW, g) was recorded on both individuals in a mating pair at the beginning and end of the feed-intake measurement. In offspring pairs, BW was measured at 21 d and 49 d of age and used to calculate average daily gain (ADG, [49-d body weight – 21-d body weight]/28 d). Percent fat and percent lean (PL, %) were predicted on culled mating pairs and 49-d-old offspring pairs using dual x-ray densitometry (PIXImus, LUNAR Corporation, 726 Heartland Trail, Madison, WI). Least-squares means were estimated for BW, ADG, and PL for each line by sex combination. Values for BW, ADG, and composition are shown in Table 4.2 for offspring and Table 4.3 for mating pairs.

Table 4.2: Body weight and composition of offspring by line[1] and sex[2] at weaning (21d of age) and harvest (49d of age) and gained for 28-d period (21d to 49d of age)

	Line			
	MH	MC	ML	Combined
Weaning Weight (g)				
Sex				
M	11.01	11.94	11.16	11.34
F	10.29	11.61	10.80	10.98
Harvest Body Weight (g)				
Sex				
M	30.69	32.76	30.31	-
F	24.43	25.70	24.01	-
Percent Lean (%)				
Sex				
M	88.6	88.0	87.8	88.1
F	87.4	86.9	86.9	87.1
Average Daily Gain (g/d)				
Sex				
M	0.70	0.74	0.68	0.71
F	0.48	0.48	0.45	0.47
Lean Gain (g)				
Sex				
M	16.31	16.05	15.56	15.97
F	12.88	13.96	11.55	12.79
Fat Gain (g)				
Sex				
M	2.02	2.17	2.12	2.10
F	1.83	2.07	1.75	1.89

[1] MH = high and ML = low heat loss selection; MC = unselected control

[2]M = male; F = female

Table 4.3: Body weight and composition of mating pairs by line[1], sex[2], and parity and percent lean at harvest

	Line			
	MH	MC	ML	Combined
Male Body Weight (g)				
Parity				
1	35.82	39.26	34.00	-
2	37.02	40.24	35.62	-
3	42.16	44.41	42.77	-
4	35.54	39.38	34.22	-
5	37.37	39.88	35.36	-
6	37.92	45.51	44.10	-
7	35.59	39.88	33.88	-
8	37.41	40.00	34.70	-
9	38.94	45.45	44.11	-
10	34.29	40.28	34.26	-
11	37.52	39.64	40.00	-
12	35.00	39.53	40.00	-
Female Body Weight (g)				
Parity				
1	53.82	52.06	46.99	-
2	50.81	51.29	51.03	-
3	48.14	53.93	46.11	-
4	56.49	51.44	47.75	-
5	54.95	53.60	49.67	-
6	47.43	54.55	47.72	-
7	55.98	54.09	45.68	-
8	51.23	56.78	46.77	-
9	46.85	55.07	51.97	-
10	55.68	49.52	47.40	-
11	58.19	54.22	40.00	-
12	54.00	55.00	45.00	-
Percent Lean				
Sex				
M	79.2	74.6	77.3	77.0
F	77.7	78.5	79.0	78.4

[1] MH = high and ML = low heat loss selection; MC = unselected control
[2]M = male; F = female

Table 4.4: Number weaned and litter weaning weight by line[1] and parity used to calculate offspring input and output.

	Line			
	MH	MC	ML	Combined
Number Weaned				
Parity				
1	11.37	10.93	9.63	10.65
2	11.40	12.26	10.96	11.54
3	10.87	11.00	9.57	10.48
4	10.88	11.02	9.73	10.55
5	9.96	10.14	9.21	9.77
6	10.07	9.37	6.87	8.77
7	8.22	9.03	6.54	7.93
8	7.18	7.26	6.42	6.95
9	5.96	6.81	6.67	6.48
10	5.52	5.82	6.15	5.83
11	5.08	4.82	2.01	3.97
12	5.00	3.80	2.00	3.00
Litter Weaning Weight (g)				
Parity				
1	113.55	117.36	101.29	110.73
2	102.57	124.32	108.99	111.96
3	111.17	114.19	101.29	108.88
4	112.30	116.41	99.63	109.45
5	102.61	115.13	94.82	104.19
6	103.15	100.39	76.12	93.22
7	88.55	107.27	69.09	88.30
8	73.02	87.00	75.23	78.42
9	64.48	76.07	73.96	71.50
10	65.85	71.07	69.94	68.95
11	64.80	58.56	21.32	48.23
12	60.00	41.80	21.00	45.00

[1] MH = high and ML = low heat loss selection; MC = unselected control

Litter traits were recorded for each pair at every parity and included number fully formed, number born alive, and number weaned (NW). Litter weaning weight (LWW) and pup weaning weight (PWW) were also recorded at 21 d of age. Least-squares means were estimated for all litter traits for each line by parity combination. Values for NW and LWW are shown in Table 4.4. Values for PWW are shown in Table 4.2.

Several culling criteria were enforced and survival analysis was performed to determine stayability of the lines. Pairs were culled due to death or illness of either member. Additionally, pairs were culled due to poor reproductive performance. If a first litter was not produced 42 d (2 full gestations lengths) after cohabitation, if they produced 2 consecutive litters with none born alive or 3 consecutive litters with none weaned, if the birth interval between consecutive parities was longer than 42 d, or if average of the most recent 2 litters was less than half the average of the first 3, then the pair was culled. Otherwise pairs were maintained, but no pairs survived 1 yr.

Survival was measured in maximum number of parities recorded for the pair before culling. Survival functions were produced for each line using Kaplan-Meier estimates (Allison, 1997). A competing risk analysis was performed to estimate the percentage of animals culled due to each culling criterion for all experimental animals and also within each line. Survival probabilities were used to estimate the parity distribution of a population of mice within each line using Markov-chain methods described by Azzam et al. (1990). This distribution was then used to produce a vector (PD) specifying the number of mating pairs at each parity in a hypothetical population of 100 total mating pairs as follows:

$$\begin{bmatrix} \pi_1 \\ \pi_2 \\ \vdots \\ \pi_i \end{bmatrix} \times 100$$

where π_i is the proportion of mating pairs at parity i. The vector, PD, was calculated assuming a maximum of 4, 8, or 12 parities. Values used for PD for each maximum scenario are shown in Table 4.5.

Table 4.5: Parity distribution, or number of mating pairs at each parity in a population of 100 mating pairs at equilibrium for each line[1], when allowing a maximum of 4, 8, or 12 parities.

	Maximum 4 Parities			Maximum 8 Parities			Maximum 12 Parities		
Line	MH	MC	ML	MH	MC	ML	MH	MC	ML
Parity									
1	27.8	25.5	26.3	15.7	13.8	14.6	12.1	10.6	11.7
2	26.5	25.1	25.1	14.9	13.6	13.9	11.5	10.4	11.1
3	24.0	25.1	24.7	13.5	13.6	13.7	10.4	10.4	11.0
4	21.7	24.3	23.9	12.2	13.2	13.3	9.4	10.1	10.6
5	-	-	-	11.7	12.7	12.9	9.0	1.0	10.3
6	-	-	-	11.3	11.7	11.6	8.7	0.9	9.3
7	-	-	-	10.9	11.0	10.9	8.4	8.4	8.7
8	-	-	-	9.7	10.3	9.0	7.5	7.9	7.2
9	-	-	-	-	-	-	6.9	6.8	6.3
10	-	-	-	-	-	-	6.0	6.4	5.0
11	-	-	-	-	-	-	5.3	5.5	4.7
12	-	-	-	-	-	-	4.8	4.8	4.2

[1] MH = high and ML = low heat loss selection; MC = unselected control

Offspring output

Offspring output was defined as lean content of offspring at 49 d and was calculated by multiplying the PD vector elementwise by the sum of the following two matrices within each line and for a maximum of 4, 8, or 12 parities:

$$\begin{bmatrix} (½NW_{P1}\text{-replacement})\times BW_M \times PL_M \\ (½NW_{P2}\text{-replacement})\times BW_M \times PL_M \\ \vdots \\ (½NW_{Pn}\text{-replacement})\times BW_M \times PL_M \end{bmatrix}$$

and

$$\begin{bmatrix} (½NW_{P1}\text{-replacement})\times BW_F \times PL_F \\ (½NW_{P2}\text{-replacement})\times BW_F \times PL_F \\ \vdots \\ (½NW_{Pn}\text{-replacement})\times BW_F \times PL_F \end{bmatrix}$$

where NW_{Pn} is the number weaned at the n^{th} parity, BW_M and BW_F are the mean body weights (kg) of 49-d-old males and females, respectively, and PL_M and PL_F are the mean percent lean content of 49-d-old males and females, respectively. Number of replacement animals was determined so that equilibrium of the PD vector would be maintained. This was accomplished by selecting enough males and females equally from all available parities to completely replace the number of parity 1 mating pairs (row 1 of the PD vector) for each maximum parity scenario. Values for number of replacement animals for each maximum parity scenario are shown in Table 4.6. Outputs were also calculated assuming NW and PL were the same across lines, because no significant differences were found due to line in previous analysis of these traits (Bhatnagar and Nielsen, 2014a, b).

The result of these calculations was a vector of lean output at each parity. All row elements were summed to obtain total lean output (kg) of offspring for each maximum parity scenario for the entire population at equilibrium.

Table 4.6: Number of female and male mating pairs saved as replacements from each available parity by line[1]

Line	MH	MC	ML
Maximum Parities			
4	6.950	6.375	6.575
8	1.963	1.750	1.825
12	1.008	0.883	0.975

[1] MH = high and ML = low heat loss selection; MC = unselected control

Offspring input

Offspring input was defined as energy intake (Mcal) for maintenance, lean growth, and fat growth of offspring from 21 to 49 d of age and was calculated by summing the following two matrices:

$$[(\sum BW_M^{0.75} \times b_{mM} + 2.9 \times lean_M + 12.8 \times fat_M)] \times PD \times \begin{bmatrix} ½NW_{P1} \\ ½NW_{P2} \\ \vdots \\ ½NW_{Pn} \end{bmatrix}$$

and

$$[(\sum BW_F^{0.75} \times b_{mF} + 2.9 \times lean_F + 12.8 \times fat_F)] \times PD \times \begin{bmatrix} ½NW_{P1} \\ ½NW_{P2} \\ \vdots \\ ½NW_{Pn} \end{bmatrix}$$

where $\sum BW_M^{0.75}$ and $\sum BW_F^{0.75}$ are the sum of the daily metabolic body weights ($kg^{0.75}$) for an individual male or female, respectively; b_{mM} and b_{mF} are energy coefficients ($kcal/kg^{0.75}$) for maintenance for males and females, respectively; $lean_M$ and $lean_F$ are mean lean gain (g) during the feeding period for males and females, respectively; and mean fat_M and fat_F are fat gain (g) during the feeding period for males and females, respectively. The values of 2.9 and 12.8 are the energy coefficients (kcal/g) for lean and

fat gain obtained from the literature (Pullar and Webster, 1977). The first part of each of the above matrices results in the energy intake of an average individual animal over the 28-d feeding period, which is then multiplied by the PD vector and ½ the mean number weaned (NW) to provide the energy intake of all males or females produced by the system and equilibrium. Sum of the daily metabolic body weights was defined as the integral of daily metabolic body weight for an individual animal over the 28-d feeding period, which was calculated for males ($\sum BW_M^{0.75}$) as follows:

$$\left[(1.75\times ADG_M)^{-1}\right]\circ[([PWW_M]+28\times[ADG_M])^{1.75}-([PWW_M])^{1.75}]$$

where ADG_M is average daily gain (kg) of an individual male offspring; PWW_M is the weaning weight (kg) of an individual male offspring at 21d of age. These calculations were repeated using values for female animals to obtain $\sum BW_F^{0.75}$. Inputs were also calculated assuming lean, fat, PWW, NW, and ADG were the same across lines, because no significant differences were found due to line in previous analysis of these traits (Bhatnagar and Nielsen, 2014a,b).

The result of these calculations resulted in a vector of the ME intake (kcal) for offspring from 21 to 49 d of age at each parity. All row elements were summed to obtain total kcal of ME intake for offspring for each maximum parity scenario for the entire population at equilibrium, which was then converted to Mcal.

Parent output

Parent output was defined as the lean output of culled parents and was calculated by element-wise multiplication of the PD vector by the percentage of culled parents (excluding those that died of natural causes) and the lean output of a mature male and

female at each parity with a 20% discount. Percentage of culled animals was calculated as follows:

$$\begin{bmatrix} Pculled_{P1} \times (100\text{-}Pdied_{P1}) \\ Pculled_{P2} \times (100\text{-}Pdied_{P2}) \\ \vdots \\ P\ culled_{Pn} \times (100\text{-}Pdied_{Pn}) \end{bmatrix}$$

where $Pculled_{Pn}$ refers to the percentage of animals culled at the n^{th} parity and $Pdied_{P1}$ refers to the percentage of animals that were lost (of the total number of animals culled) due to death or illness rather than reproductive reasons. Values for total percentage culled and percentage lost (of total culled) due to death or illness are shown in Table 4.7. The lean output was then calculated as:

$$\begin{bmatrix} BW_{MP1} \\ BW_{MP2} \\ \vdots \\ BW_{MPn} \end{bmatrix} \times PL_M + \begin{bmatrix} BW_{FP1} \\ BW_{FP2} \\ \vdots \\ BW_{FPn} \end{bmatrix} \times PL_F$$

where BW_{MPn} and BW_{FPn} are body weights (kg) of a male and female, respectively weaning of their n^{th} parity, and PL_M and PL_F are percent lean of adult males and females, respectively. Outputs were also calculated assuming PL was the same across lines, since no significant differences were found due to line in previous analysis (Bhatnagar and Nielsen, 2014a).

The result of these calculations was a vector of lean output of culled mating pairs at each parity, which was multiplied by 0.80 (20% discount). All row elements were summed to obtain total lean output (kg) of culled mating pairs for each maximum parity scenario for the entire population at equilibrium.

Table 4.7: Total percentage of mating pairs culled and percentage (of total culled) lost due to death or illness at each parity by line[1,2]

	Total Percentage Culled (%)			Percentage Culled due to Death or Illness (%)		
Line	MH	MC	ML	MH	MC	ML
Parity						
1	4.80	1.60	4.80	0.10	0.10	0.10
2	9.50	0.00	1.60	0.30	0.20	0.30
3	9.50	3.20	3.20	0.70	0.40	0.60
4	4.80	3.20	3.20	0.80	0.40	0.70
5	3.20	7.90	9.50	1.30	0.70	1.10
6	3.20	6.30	6.30	1.40	0.80	1.20
7	11.1	6.30	17.5	2.30	1.20	2.00
8	7.90	14.3	12.7	3.00	1.60	2.50
9	12.7	4.80	20.6	3.50	1.90	3.00
10	11.1	14.3	6.30	4.10	2.20	3.50
11	9.50	12.7	9.50	5.10	2.70	4.40
12	100	100	100	6.30	3.40	5.10

[1] MH = high and ML = low heat loss selection; MC = unselected control
[2]Assuming a maximum of 12 allowed parities; for scenarios assuming 4 or 8 maximum parities, columns would be truncated and 100% of remaining mating pairs are culled at 4 or 8 parities

Parent input

Parent input was defined as energy intake (Mcal) for mating pairs and was calculated by multiplying the PD vector by metabolizable energy intake for maintenance and weaning weight during the entire feeding period. Intake was calculated as follows:

$$\begin{bmatrix} BW_{P1}{}^{0.75} \\ BW_{P2}{}^{0.75} \\ \vdots \\ BW_{Pn}{}^{0.75} \end{bmatrix} \circ \begin{bmatrix} b_{mj} \\ b_{mj} \\ \vdots \\ b_{mj} \end{bmatrix} \circ \begin{bmatrix} dof_1 \\ dof_2 \\ \vdots \\ dof_n \end{bmatrix} + 5.28 \times \begin{bmatrix} LWW_{P1} \\ LWW_{P2} \\ \vdots \\ LWW_{Pn} \end{bmatrix}$$

where BW_{Pn} is the sum of male and female metabolic body weights ($kg^{0.75}$) for mating pairs at the n^{th} parity, b_{mj} is the energy coefficient for maintenance ($kcal/kg^{0.75}$) at the j^{th} feeding period that corresponds to the n^{th} parity (parities 1-3 were feeding period 1,

parities 4-6 were feeding period 2, etc.), dof_1 is the mean number of days on feed for a mating pair at each period (values for dof are shown in Table 4.8), and LWW_{Pn} is the mean litter weaning weight (g) at the n^{th} parity. The value of 5.28 comes from previous analysis of this population and is the energy coefficient (kcal/g) which encompasses energy of lactation and feed intake of the litter prior to weaning. Inputs were also calculated assuming LWW was the same across lines, because no significant differences were found due to line in previous analysis of these traits (Bhatnagar and Nielsen, 2014b).

Table 8: Days on feed for mating pairs for each parity by line[1]

	Line		
	MH	MC	ML
Parity			
1	42.90	42.90	42.81
2	24.44	25.26	25.50
3	25.28	25.81	27.00
4	25.23	25.18	24.72
5	23.12	22.40	22.65
6	23.64	24.81	23.93
7	24.53	24.63	24.40
8	21.85	23.67	19.25
9	30.20	28.32	30.95
10	16.55	27.76	27.10
11	22.77	29.87	25.02
12	20.01	20.56	20.03

[1] MH = high and ML = low heat loss selection; MC = unselected control

The result of these calculations resulted in a vector of the ME intake (kcal) for mating pairs during gestation and lactation at each parity. All row elements were summed

to obtain total kcal of ME intake for mating pairs for each maximum parity scenario for the entire population at equilibrium, which was then converted to Mcal.

Lifecycle efficiency

After calculating all inputs and outputs, lifecycle biological efficiency was then calculated for each line and maximum parity scenario as follows:

$$\frac{\text{(Offspring Output + Parent Output)}}{\text{(Offspring Input + Parent Input)}}.$$

Efficiency was calculated with inputs and outputs utilizing all line specific differences as well as when only traits shown to be significantly different were used.

Results

Offspring output and input

Output and input of offspring when designating a maximum of 4, 8, or 12 parities are shown in Fig. 4.1. Control mice offspring produced the greatest amount of output, while ML mice produced the least amount of output and MH were intermediate. MC mice produced 8 to 10% more output than MH mice and 23 to 26% more output than ML mice, depending on the maximum number of allowed parities. Output was greatest when allowing a maximum of 8 parities for all lines, and similar at a maximum of 4 and 12 parities, showing a slight quadratic effect. When output was calculated assuming lines had identical NW and PL, offspring output was similar between MH and ML mice, while MC mice still showed greatest output (Fig. 4.2). The same quadratic trend for number of parities was observed for all lines.

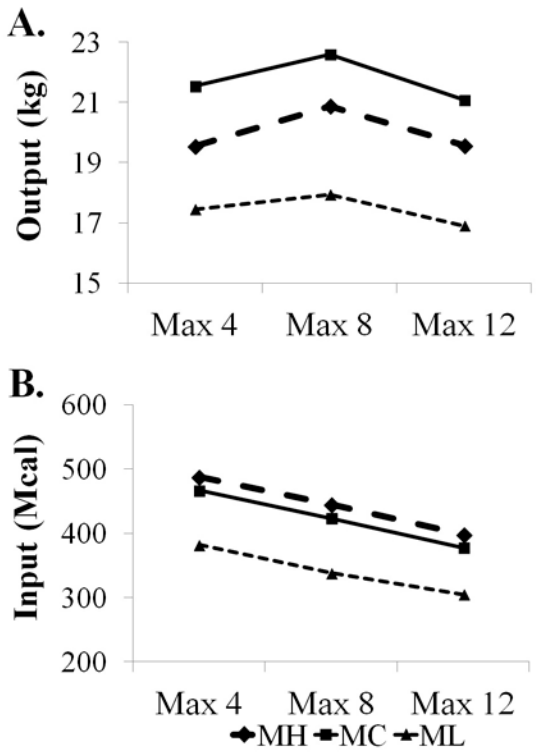

Figure 4.1. Lean body weight (kg) output (panel A) and feed intake (Mcal) input (panel B) for offspring of mice from lines selected for high (MH) or low heat loss (ML) or unselected control (MC) when held to a maximum of 4, 8, or 12 allowed parities

Offspring from the MH line had the largest input in all maximum parity scenarios, consuming 25 to 32% more energy than ML offspring and 5% more energy than MC offspring, depending on the maximum number of allowed parities. Inputs decreased when the maximum number of allowed parities was increased for all lines. When output was calculated assuming lines had identical lean, fat, PWW, and ADG, MH still had the largest input of the lines, but MC and ML were nearly identical (Fig. 4.2). The same decrease was observed when maximum number of allowed parities was increased.

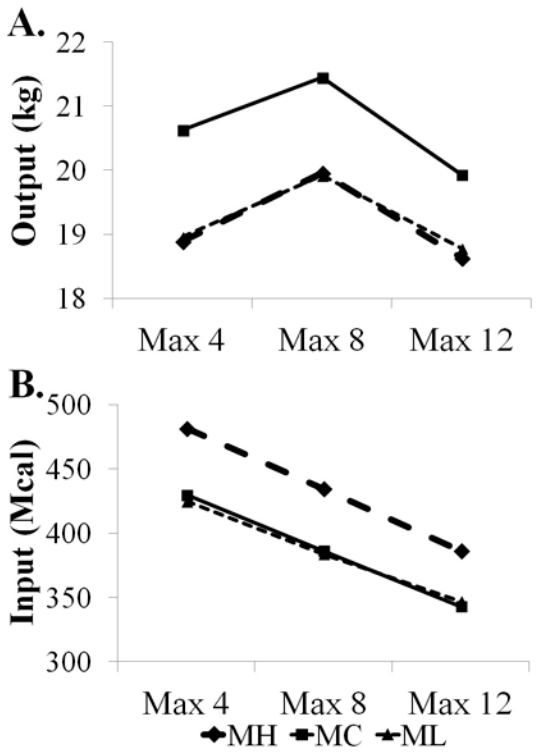

Figure 4.2. Lean body weight (kg) output assuming number weaned and percent lean were constant across lines (panel A) and feed intake (Mcal) input assuming lean, fat, weaning weight, and average daily gain were constant across lines (panel B) for offspring of mice from lines selected for high (MH) or low heat loss (ML) or unselected control (MC) when held to a maximum of 4, 8, or 12 allowed parities.

Parent output and input

Output and input of mating pairs when designating a maximum of 4, 8, or 12 parities are shown in Fig. 4.3. Output was much smaller for mating pairs than offspring, as it only included output from culled parents. Largest output came from MH mice, particularly when a maximum of 4 parities was enforced. MH mice produced 6 to 11% more output than MC mice and 8 to 18% more output than ML mice, depending on the maximum number of allowed parities. Assuming PL was the same for all three lines did not drastically change parent output. Output was more similar between MC and MH mice at a maximum of 4 allowed parities (Fig. 4.4).

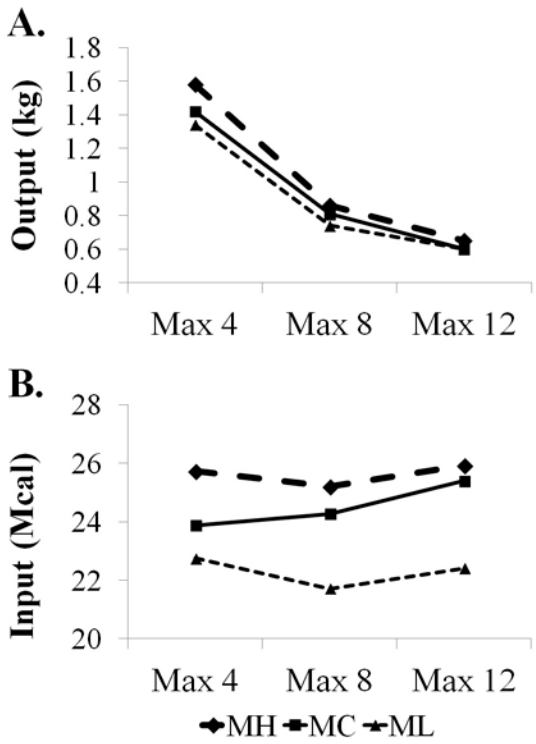

Figure 4.3. Lean body weight (kg) output (panel A) and feed intake (Mcal) input (panel B) for mating pairs of mice from lines selected for high (MH) or low heat loss (ML) or unselected control (MC) when held to a maximum of 4, 8, or 12 allowed parities.

As expected, MH mating pairs had the largest input while ML had the lowest and MC was intermediate (Fig 4.3). MH pairs consumed 2 to 8% more energy than MC pairs and 16 to 18% more energy than ML pairs, depending on the maximum number of allowed parities. For MH and ML mating pairs, there was a slight decrease in inputs when the maximum number of parities allowed was increased from 4 to 8, followed by an increase at a maximum of 12 parities. However, inputs for MC mating pairs increased as maximum number of parities increased, so that there was only a 2% difference between MC and MH mating pairs when allowing a maximum of 12 parities. When LWW was assumed to be the same across lines, parental input was similar to when LWW was varied due to line (Fig. 4.4).

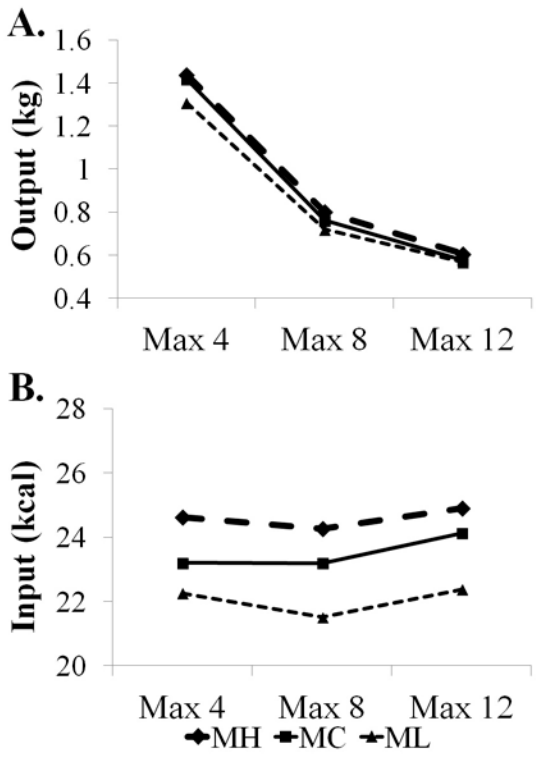

Figure 4.4. Lean body weight (kg) output assuming percent lean was constant across lines (panel A) and feed intake (Mcal) input assuming weaning weight was constant across lines (panel B) for mating pairs of mice from lines selected for high (MH) or low heat loss (ML) or unselected control (MC) when held to a maximum of 4, 8, or 12 allowed parities.

Lifecycle efficiency

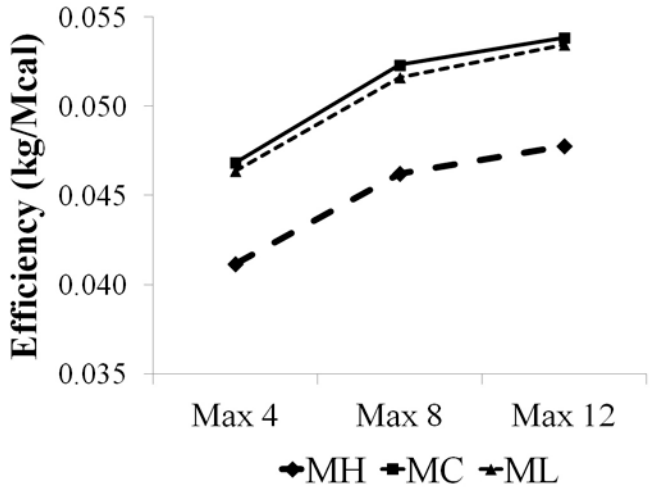

Figure 4.5. Lifecycle biological efficiency (total offspring and mating pair output divided by total offspring and mating pair input) of mice selected for high (MH) or low heat loss (ML) or unselected control (MC) when held to a maximum of 4, 8, or 12 allowed parities.

Lifecycle biological efficiency of each line is shown in Fig. 4.5 at a maximum of 4, 8, or 12 parities. The MC line showed the most superior efficiency in all scenarios due to greater offspring output and moderate performance in all other categories, but MC mice were not appreciably more efficient than ML mice. Control mice were 13 to 14% more efficient than MH mice, but only 1 to 2% more efficient than ML mice. Substantially reduced intake by the ML line resulted in improved efficiency over the MH line, (12 to 13%) but reduced output did diminish some of the benefit. Efficiency increased in all lines as maximum number of parities allowed increased from 4 to 8, and continued to increase when a maximum of 12 parities was allowed, though at a slower rate. When lifecycle efficiency was calculated using input and output values determined with the same values across lines for traits that were not significantly different due to line, efficiency values did not change substantially, though the advantage of the MC line over the MH line was slightly more pronounced, and the MC line was more efficient than

the ML line (Fig. 4.6). The MC line was 19 to 21% more efficient than the MH line and 7% more efficient than the ML line.

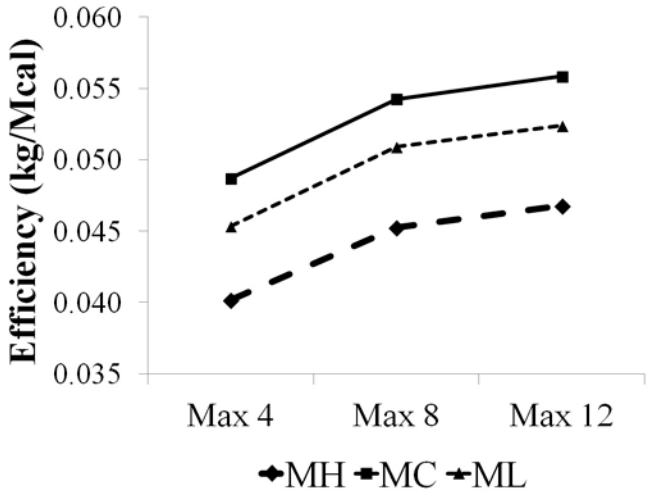

Figure 4.6. Lifecycle biological efficiency (total offspring and mating pair output divided by total offspring and mating pair input) of mice selected for high (MH) or low heat loss (ML) or unselected control (MC) when held to a maximum of 4, 8, or 12 allowed parities using input and output values that assumed number weaned, weaning weight, percent lean, average daily gain, lean gain and fat gain were constant across lines.

Discussion

The increased output observed in MC offspring was due to larger body weights and larger litter sizes, compared to either selection line (Bhatnagar and Nielsen, 2014a,b). Heavier body weights in MC mice have been consistently shown in this population (McDonald and Nielsen, 2007; Nielsen et al., 1997a). Though not found to be significant, ML mice did have slightly smaller litter sizes, body weights, and lean content compared to MH mice and this accounts for the lower levels of output seen in ML offspring compared to MH offspring (Bhatnagar and Nielsen, 2014a,b). Using the same values for NW and PL confirms the importance of these factors in total output as the difference between MH and ML mice is essentially eliminated. The impact of increased BW in MC

is also evident as MC mice still have the largest output, even when NW and PL were assumed identical across lines. The drop in offspring output from maximum of 8 to maximum of 12 parities is likely due to smaller litter sizes from older mating pairs, as litter size was found to significantly decrease in later parities for all lines (Bhatnagar and Nielsen, 2014b).

The greater input of MH offspring was expected considering the extensive previous work in these lines showing greater intake of MH mice, though previously MH mice consumed much more than MC mice than what was observed in this study (McDonald and Nielsen, 2007; Nielsen et al., 1997a). Larger litter size in MC mice is the likely explanation for the similarity between MH and MC mice, as the greater number of pups born to MC mice require more input. The similarity between ML and MC mice when lean, fat, PWW, NW, and ADG are constant across lines is likely due to PWW and NW. Eliminating the effects of slightly smaller litter sizes and weaning weights of ML mice in calculations diminished the reduced intake of offspring in the ML line. Inputs decreased as maximum number of parities increased due to smaller litter sizes from older mating pairs (Bhatnagar and Nielsen, 2014b).

Increased parent output in MH mice was due to poorer survival rates of MH mice at early parities compared to ML or MC mice, resulting in more mating pairs being culled (Bhatnagar and Nielsen, 2014b). Differences in PL between MC and MH were also partially responsible, demonstrated by the reduced differences in output when PL was assumed constant across lines. Output from ML mice was lowest due to slightly smaller body weights and lean content compared to the other two lines (Bhatnagar and Nielsen, 2014a). The drop in output from the maximum of 4 parities to maximum of 8 parities

scenario is due to the fact that all remaining pairs are culled at the final parity, regardless of reproductive performance. In the maximum of 4 parities scenario, fewer pairs have been previously culled since survival rates are generally high in the younger animals (Bhatnagar and Nielsen, 2014b). Therefore, a larger number of animals were in this scenario. For example, 25.4 MC mating pairs were culled when only allowing 4 parities, while only 13.6 pairs were culled when allowing 8 parities.

As expected, parent inputs were greatest for the MH line due to the greater feed intake consistently observed in mice from this line (Bhatnagar and Nielsen, 2014a; McDonald and Nielsen, 2007; Nielsen et al., 1997a). Holding LWW constant across lines had little effect on input, most likely since offspring account for a small percentage of total intake compared to the intake of the parents. Authors cannot account for the slightly quadratic nature of MH and ML parent input when increasing maximum number of allowed parities. Input would be expected to increase as maximum number of parities increased, as seen in the MC line, since this would increase days on feed.

Authors hypothesized that the improvement in feed intake observed in ML mice would carry throughout a lifecyle and not be outweighed by any correlated responses in output traits, however this was not entirely confirmed in this study. While ML mice did have greater overall efficiency when compared to MH mice, ML and MC mice were very similar in terms of biological efficiency. The substantially increased offspring output in MC mice was primarily responsible for the greatest overall efficiency observed in the MC line. Even when litter and body composition traits were held constant across lines, the increased BW of MC lines was not overcome, and the greater efficiency of the MC line was enhanced. In fact, holding litter size constant reduced input for MC mice so that

it was similar to ML mice. This resulted in superior efficiency in the MC line. Previous work in rats has observed an improvement in lifecycle efficiency in animals selected for rate of lean growth or efficiency of lean growth compared to control animals (Wang and Dickerson, 1984). The improvement was greatest in the lean growth line due to the increased growth rate, lower metabolic rate, and larger litter size observed in those animals.

Lifecycle efficiency was shown to increase at similar rates as maximum number of allowed parities increased in all lines. The increase in efficiency as maximum number of parities allowed increased was likely due to the corresponding decrease in offspring input and a decrease in offspring output due to smaller litter sizes from a maximum of 8 to a maximum of 12 parities. Results suggest that maintaining breeding animals for a greater number of parities maximizes output and improves efficiency, at least in litter-bearing species. Previous studies in lifecycle efficiency have found that reproductive longevity allows the cost of maintaining parents to be somewhat mediated by spreading inputs over increased output (Newman et al., 1985; Wang and Dickerson, 1984)

Increased offspring output appears to have the largest influence on lifecycle efficiency in this evaluation, as it accounts for about 95% of the total output of the system. Litter size and body weight most likely have the largest impact on offspring output, with survival rates having a smaller effect. The importance of litter size to lifecycle efficiency has been observed in several other studies utilizing a rodent model. Newman et al. (1985) measured lifetime production in several crosses of mouse lines. Number of offspring produced, which would largely be affected by litter size and number of litters produced, was found to have a substantial effect on lifecycle efficiency, both

from the input and output side. Larger body weights in rats selected for increased lean growth was responsible for increased outputs and improved efficiency observed in those animals (Wang and Dickerson, 1984). The lean gain line also had improved maternal performance, which was also responsible for the observed improvement in lifecycle efficiency. Improvements in litter traits and growth rates were also found to be the most important factors for improved lifecycle efficiency in swine (Tess et al., 1983).

In beef cattle systems, interpretations of lifecycle efficiency studies are much different from rodent or swine studies. In a majority of these studies, feed intake, particularly of the dam, was one of the most important factors affecting efficiency (Davis et al., 1994; Davis et al., 1983b; Naazie et al., 1999). Reproductive performance was only important in terms of reproductive longevity so that cows will produce more calves and therefore increase outputs (Davis et al., 1983a). Multiple studies also found a greater benefit in reducing maintenance energy requirements in beef cattle than in litter bearing species, because reducing feed intake has a larger benefit to improving economic efficiency (Montano-Bermudez and Nielsen, 1990; Naazie et al., 1999).

Ultimately, selection for reduced maintenance energy requirements should be investigated further to determine its usefulness in a livestock production system. For swine production, reducing maintenance energy requirements may be a useful avenue in developing terminal sire lines, where reproductive performance is less important and reducing feed intake would be more beneficial. For maternal lines, maintenance energy requirements could be used as part of a selection index with reproductive performance, to balance potential detrimental effects. It is difficult to make direct comparisons between mice and cattle due to reproductive difference, but previous studies in lifecycle efficiency

have shown that reducing inputs is more important in cattle than in litter bearing species. Potentially, reducing maintenance energy in cattle would be more likely to improve lifecycle biological efficiency, though the effects of such reduction on cattle reproductive performance are unclear. Additionally, it may be useful to evaluate the lifecycle economic efficiency of these lines, since MC and ML lines are similar in terms of efficiency, but ML mice require significantly less inputs.

Implications

Selection to reduce maintenance energy requirements in mice has been proven successful and is correlated with a reduction in feed intake that is maintained when modeled in a lifecycle production system. However, small detrimental changes in reproductive performance, body composition, and survival rates were found in low maintenance animals. In this study, authors show that reduced output offsets the benefit of reduced feed intake in terms of lifecycle biological efficiency and this result was highlighted when litter and body composition traits were held constant across lines. While low maintenance animals had a more desirable efficiency than high maintenance animals, improved outputs resulted in similar efficiency between control and low maintenance animals. Therefore, selection to reduce maintenance energy requirements should be considered with some reservation. The reduction in feed efficiency could make it a useful trait in terminal crosses where reproductive performance and survival rates are less important, or as part of a selection index. Additionally, lifecycle economic efficiency should be evaluated. Ultimately, the best strategy for improving lifecycle efficiency remains an important issue for livestock species.

Literature cited

Allison, P. D. 1997. Survival analysis using the SAS system. A practical guide. SAS Institute Inc., Cary, NC, USA.

Azzam, S. M., A. M. Azzam, M. K. Nielsen, and J. E. Kinder. 1990. Markov chains as a shortcut method to estimate age distributions in herds of beef cattle under different culling strategies. J. Anim. Sci. 68: 5-14.

Bhatnagar, A. S., and M. K. Nielsen. 2014a. Body composition and feed intake of reproducing and growing mice divergently selected for heat loss. J. Anim. Sci. in press

Bhatnagar, A. S., and M. K. Nielsen. 2014b. Lifetime reproductive performance and survival analysis of mice divergently selected for heat loss. J. Anim. Sci. in press

Davis, K. C., M. W. Tess, D. D. Kress, D. E. Doornbos, and D. C. Anderson. 1994. Life cycle evaluation of five biological types of beef cattle in a cow-calf range production system: II. Biological and economic performance. J. Anim. Sci. 72: 2591-2598.

Davis, M. E., J. J. Rutledge, L. V. Cundiff, and E. R. Hauser. 1983a. Life cycle efficiency of beef production: I. Cow efficiency ratios for progeny weaned. J. Anim. Sci. 57: 832-851.

Davis, M. E., J. J. Rutledge, L. V. Cundiff, and E. R. Hauser. 1983b. Life cycle efficiency of beef production: II. Relationship of cow efficiency ratios to traits of the dam and progeny weaned. J. Anim. Sci. 57: 852-866.

McDonald, J. M., and M. K. Nielsen. 2007. Renewed selection for heat loss in mice: Direct responses and correlated responses in feed intake, body weight, litter size, and conception rate. J. Anim. Sci. 85: 658-666.

Montano-Bermudez, M., and M. K. Nielsen. 1990. Biological efficiency to weaning and to slaughter of crossbred beef cattle with different genetic potential for milk. Journal of Animal Science 68: 2297-2309.

Naazie, A., M. Makarechian, and R. J. Hudson. 1999. Evaluation of life-cycle herd efficiency in cow-calf systems of beef production. J. Anim. Sci. 77: 1-11.

Newman, S., D. L. Harris, and D. P. Doolittle. 1985. Economic efficiency of lean tissue production through crossbreeding: Systems modeling with mice. II. Reproduction-growth termination alternatives. J. Anim. Sci. 60: 395-412.

Nielsen, M. K., B. A. Freking, L. D. Jones, S. M. Nelson, T. L. Vorderstrasse, and B. A. Hussey. 1997a. Divergent selection for heat loss in mice: II. Correlated responses in feed intake, body mass, body composition, and number born through fifteen generations. J. Anim. Sci. 75: 1469-1476.

Nielsen, M. K., L. D. Jones, B. A. Freking, and J. A. DeShazer. 1997b. Divergent selection for heat loss in mice: I. Selection applied and direct response through fifteen generations. J. Anim. Sci. 75: 1461-1468.

Pullar, J. D., and A. J. F. Webster. 1977. The energy cost of fat and protein deposition in the rat. Brit. J. Nutr. 37: 355-363.

Tess, M. W., G. L. Bennett, and G. E. Dickerson. 1983. Simulation of genetic changes in life cycle efficiency of pork production, II. Effects of components on efficiency. J. Anim. Sci. 56: 354-368.

Wang, C.-T., and G. E. Dickerson. 1984. Selection for rate and for efficiency of lean growth in rats: Net life-cycle efficiency. J. Anim. Sci. 59: 49-66.

Williams, C. B., and T. G. Jenkins. 2003. A dynamic model of metabolizable energy utilization in growing and mature cattle. I. Metabolizable energy utilization for maintenance and support metabolism. J. Anim. Sci. 81: 1371-1381.

APPENDIX A

Markov chain estimation of parity equilibrium

Markov-chain methodology was used to estimate proportions of mating pairs at each parity for a population at equilibrium by using a transition matrix (P) to solve the following system of equations $\pi_i = P\pi_i$. The transition matrix relates the probability a mating pair at parity i will be retained in the population to become a mating pair at parity i + 1, or if they would be replaced by a parity-1 mating pair. Probabilities were taken from values estimated in the survival analysis. The value of π_i refers to a vector of the proportion of mating pairs in the population at each parity i, when the distribution has reached equilibrium, assuming population size is constant. Parity distributions were calculated for each line assuming animals were maintained a maximum of 4, 6, 8, 10, or 12 parities, or for 1 yr as was done in the study. An example set of equations for MH mating pairs assuming a maximum of 4 parities is shown below, where the probability of a parity-1 mating pair advancing to parity 2 is 0.952, the probability of a parity-2 mating pair advancing to parity 3 is 0.905, and the probability of a parity-3 mating pair advancing to parity 4 is 0.905:

$$\begin{bmatrix}\pi_1\\ \pi_2\\ \pi_3\\ \pi_4\end{bmatrix} = \begin{bmatrix}0.048 & 0.095 & 0.095 & 1\\ 0.952 & 0 & 0 & 0\\ 0 & 0.905 & 0 & 0\\ 0 & 0 & 0.905 & 0\end{bmatrix}\begin{bmatrix}\pi_1\\ \pi_2\\ \pi_3\\ \pi_4\end{bmatrix}$$

Then, the solution to these equations follows as:

$$\pi_i = P\pi_i$$

$$0 = P\pi_i - I\pi_i$$

$$0 = (P - I)\,\pi_i$$

which, in matrix form, results in the following:

$$\begin{bmatrix}0\\0\\0\\0\end{bmatrix} = \begin{bmatrix}-0.952 & 0.095 & 0.095 & 1\\0.952 & -1 & 0 & 0\\0 & 0.905 & -1 & 0\\0 & 0 & 0.905 & -1\end{bmatrix}\begin{bmatrix}\pi_1\\\pi_2\\\pi_3\\\pi_4\end{bmatrix}$$

In its current form, this set of equations has no direct solution, however this can be corrected by eliminating one equation and replacing it with $\Sigma_i \pi_i = 1$, or the sum of the probabilities is equal to 1, as shown below:

$$\begin{bmatrix}1\\0\\0\\0\end{bmatrix} = \begin{bmatrix}1 & 1 & 1 & 1\\0.952 & -1 & 0 & 0\\0 & 0.905 & -1 & 0\\0 & 0 & 0.905 & -1\end{bmatrix}\begin{bmatrix}\pi_1\\\pi_2\\\pi_3\\\pi_4\end{bmatrix}$$

The set of equations can then be easily solved by Gaussian elimination, or using a generalized inverse, resulting in:

$$\begin{bmatrix}\pi_1\\\pi_2\\\pi_3\\\pi_4\end{bmatrix} = \begin{bmatrix}0.27\\0.26\\0.24\\0.22\end{bmatrix}$$

Thus at equilibrium, the proportion of mating pairs at parity 1, 2, 3 and 4 are 0.27, 0.26, 0.24, and 0.22, respectively.

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